Individual differences in maternal behaviour in the grey seal (Halichoerus grypus) and the impact of disturbance at Donna Nook.

JAMES, HANI,MYFANWY,CASTLE

Individual differences in maternal behaviour in the grey seal (*Halichoerus grypus*) and the impact of disturbance at Donna Nook.

Hani Myfanwy Castle James

Thesis submitted for the degree of
Master of Science (by research)

School of Biological and Biomedical Sciences

Durham University

2012

Individual differences in maternal behaviour in the grey seal (*Halichoerus grypus*) and the impact of disturbance at Donna Nook.

Hani Myfanwy Castle James

ABSTRACT

The grey seal (*Halichoerus grypus*) is a semi-aquatic pinniped and a member of the family phocidae. On shore breeding colonies form around the UK during the autumn, on which female grey seals rear a single pup for up to 3 weeks. This study aimed to identify the presence, if any, of consistent individual differences (CIDs) in maternal behaviour of grey seals on the breeding colony at Donna Nook. Data collection was non-intrusive, using in-field focal videos, pup-check counts and proximity maps. In agreement with previous research on North Rona, results from the current study show CIDs in pup-check behaviour at Donna Nook. CIDs were present in time mothers spent interacting with their pup and time spent nursing. These findings provide preliminary evidence for the existence of grey seal personalities, or more specifically, mothering styles. There was no evidence that pup gender influenced maternal behavioural investment. There was evidence for habituation to human disturbance; females exposed to the public showed a lower duration of alert behaviour and no difference in duration of pup interaction compared to the restricted site. This habituation was no longer evident following exposure to sudden disturbance (in this case, an unforeseen tidal storm surge). A colony wide increase in pup-check duration and decrease in colony density was observed in the weeks following the storm surge. The results of this study highlight the scope for future research into the stability of these CIDs over numerous breeding seasons and effects of these differences on pup development. Perhaps most importantly, this study provides the first photographic identification catalogue for females at Donna Nook, an excellent basis for future research.

DECLARATION OF COPYRIGHT

I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any other university. In all cases, where it is relevant, material from the work of others has been acknowledged.

Hani Myfanwy Castle James

School of Biological and Biomedical Sciences

Durham University

2012

CONTENTS

LIST OF TABLES

LIST OF FIGURES

5. DISCUSSION AND INTERPRETATION

ACKNOWLEDGEMENTS

To begin, I would like to thank my supervisor, Dr Sean Twiss. His knowledge, support, guidance and calm attitude have been invaluable throughout the course of this project. My thanks also to the Lincolnshire Wildlife Trust wardens at Donna Nook for their hospitality throughout the fieldwork season, in particular Rob Lidstone-Scott for his expert advice and colony counts! I am sincerely grateful to Maz Castle, Jon James and Charles and Betty Castle for providing the support, both financial and emotional, necessary to make this project possible. And finally, to Stephen Long for both his encouragement and the tireless hours of proof reading.

1. INTRODUCTION

Variation in the behaviour of species, or populations within species, is well understood. Geographical separation or different physiological states experienced by populations can cause an overall change in behaviour of the members to suit these different conditions (Dall *et al*., 2004). However, throughout current literature we are seeing individuals within the same population and under the same (or similar) conditions behaving differently. These behavioural differences cannot be explained by gender, age, body size or external conditions alone (Wilson *et al*., 1994; Wolf & Weissing, 2010). Mechanisms behind these differences are still under contention; individuals' behaviours appear to be consistent, sometimes throughout their entire lifecycle, relative to one another. We, as humans, are beginning to accept that we may not be the only species with personalities.

There are numerous benefits to be gained from our understanding of non-human personality, both for ourselves and for the study species. Decisions regarding animal welfare now take into account the personality of individuals. For example, rehoming centres can use animal personality assessments to identify the best type of home for a pet (Gosling, 2008). Similarly, zoos are able to select the most appropriate course of care, such as a type of environmental enrichment, for an individual's needs, based on their personality type (Watters & Powell, 2012). Benefits of understanding animal personalities for the human race come in a number of forms. Measures of personality can save us time, effort and money when using working animals. For example, personality scores are now given to detector dogs two weeks into their training regime. Traits selected reflect the individual's overall desire to work: almost 100% of those scoring high after two weeks were found to pass their final detection test four months later; of those scoring low only half passed the final examination. Using personality score data, training schemes are able to remove dogs from the programme which receive low scores after two weeks. This means fewer resources are used training dogs with low chances of success, and the failure rate at the end of the course is reduced (Gosling, 2008). Behaviours similar to our own have been identified even in invertebrate species. For example, behaviours related to anxiety and caution have been observed in planarian worms. As a result of these discoveries, research has seen movement away from 'psychological state' in animals, and towards acceptance of animal personalities much like our own (Gosling, 2001). Though comparisons must be made carefully, taking

into account the ecology and social nature of the species, animals are now being used as models to answer questions about human personality. Animals can be manipulated, placed into unnatural environments and kept under more controlled circumstances than would be deemed acceptable for human subjects. Many species have shorter life-cycles than our own and can be used to answer questions about the ontogeny and development of behaviour (Gosling, 2001 & 2008).

This chapter discusses the solid and ever expanding foundation of non-human personality research, as well as non-human mothering styles (an extension of personality focussing wholly on maternal behaviours). Evidence is given for both, and comparisons drawn with human personalities.

1.1 DEFINING PERSONALITIES

The term personality has become common in the study of human psychology. Thedefinition given in psychology literature is "Underlying behavioural tendencies that differ across individuals, that are consistent within individuals over time, and that affect the behaviour that is expressed in different contexts." (Stamps & Groothius, 2010). Awareness of the existence of personalities in non-human species began as early as Pavlov's studies on canine behaviour. Pavlov identified four basic personality traits from the different characteristics appearing in dogs (*Canis lupus familiaris*) in response to novel test environments and stimuli (Pavlov, 1960). Pavlov used ancient classifications (sanguine, melancholic, phlegmatic, choleric), previously used only in human psychology, to define the differences in characteristics he was observing. He was, in fact, describing individual differences in the expression of personality traits linked with novel environment exploration, now known as shyness, boldness, exploration and avoidance (Réale *et al*., 2007). Despite this pioneering work over a century ago (published initially in 1901), non-human personalities have only begun to receive extensive research attention in the last two decades. Several terms are now employed in non-human personality literature, such as coping styles and behavioural syndromes (these, along with other key terms, are defined in **Table 1.1.1**) Whilst differing slightly in their overall meaning, all of these terms refer to a set of consistent individual differences (CIDs) within a given population (Sih *et al*., 2004; Dingemanse *et al*., 2010). The differentiation comes when considering whether this consistency is measured across time periods, across contexts (defined in **Table 1.1.1**) or both. It appears commonplace in literature now to accept sets of CIDs as evidence of 'personalities' only when these have been shown to be repeatable across both (Stamps, 2007; Dingemanse *et al*., 2010; Stamps & Groothuis, 2010). However, there is good support for using the term 'personality' as a blanket term for a set of CIDs, so long as these consistencies exist across time. The reasons for this are summarised by Gosling (2008): it is preferable to keep terminology to a minimum where there is little requirement to complicate matters, to allow comparison between human and non-human literature, and to ensure no assumptions are made about non-human personalities simply due to an overlap in terminology with human studies. As such, Gosling offers a more inclusive definition of animal personality to cover these differences:

"*Those characteristics of individuals that describe and account for temporally stable patterns of affect, cognition and behaviour*" (Gosling, 2008).

Table 1.1.1 Definition of key terms related to the identification of CIDs

Term	Definition	Source
Behavioural definitions		
Consistent Individual Differences	"Individual differences in behaviour that are consistent over time (within or across generations) and/or different contexts"	Dall *et al*., 2004
Personality	"Consistent differences between individuals in their behaviour across time and contexts." "Those characteristics of individuals that describe and account for temporally stable patterns of affect, cognition and behaviour"	Dingemanse *et al*., 2010 Gosling, 2008
Behavioural Syndrome	"A suite of correlated behaviours reflecting between individual consistency in behaviour across multiple situations." "...involves both within- and between-individual consistency in behaviour"	Sih *et al*., 2004 Luttbeg & Sih, 2010
Coping Style	"The suite of behavioural and physiological responses of an individual that characterise its reactions to a range of stressful situations." This definition distinguishes between individuals in two groups - reactive copers which respond to their external environment, and proactive copers who respond less.	Dingemanse *et al*., 2010
Condition definitions		
State	"Those features of an organism (eg. morphological, physiological, neuro-biological or environmental) that affect the balance between the costs and benefits of its behavioural actions"	Dingemanse & Wolf, 2010
Situation	"A given set of conditions at one point in time" either environmental or physiological	Sih *et al*., 2004
Context	"A functional behavioural category; eg. feeding, mating, antipredator, parental care, contest or dispersal contexts."	Sih et al., 2004
Plasticity definitions		
Adaptive phenotypic plasticity	"The potential for an organism to produce a range of different, relatively fit phenotypes in multiple environments….the ability to produce a better phenotype-environment match across more environments than would be possible by producing a single phenotype"	DeWitt *et al*., 1998
Behavioural Plasticity	The ability of organisms to express a range of behaviours which are "unlimited, immediate and infinitely reversible"	Sih *et al*., 2004
Developmental Plasticity	Includes induced changes in morphology, physiology or life histories. "Thought to unfold slowly and be irreversible"	Sih et al., 2004

As stated, personality is the summation of a set of CIDs in behavioural traits. The typical approach to studying CIDs requires the identification of behavioural traits related to key behavioural axes. The main behavioural axes studied are shy-bold; exploration-avoidance; aggression; activity and sociability (Réale *et al.,* 2007). These can be compared from individual to individual in a population and, when CIDs are present, individuals' relative positions on these axes remain consistent; put simply, "Each individual does not express the full range of behavioural trait values present in its population" (Dingemanse *et al*., 2010). However, this does not mean that an individual behaves consistently with relation to itself; trait values may fluctuate along these axes depending on the context the animal finds itself in. Instead it means that the differences between two or more individuals' behaviours remain consistent during these fluctuations; known as stable behavioural variation (McElreath & Strimling, 2006; Wolf & Weissing, 2010). These between-individual differences are unrelated to environmental influence, sex, age or body size (Wilson *et al.,* 1994; Wolf & Weissing, 2010), and are therefore a product of some intrinsic determinant of the individual's behaviour.

1.1.1 Measuring consistent differences

Behavioural consistency can be measured between two or more time periods (temporally) or two or more contexts. Consistent differences are shown when an individual maintains a consistent position on the axis of a behavioural trait relative to other individuals in the same population, either across time periods, contexts or both. All definitions in this section are taken from Stamps & Groothuis (2010). Note, depending on the definition of time period, different contexts inevitably occur at different time periods. A single time period could cover only a short section of one context (for example one day of the breeding season), similarly one time period may account for the entirety of one context. Rarely, a time period covers the entire life cycle. This allows for trans-generational comparison. However, more often than not, individual contexts are considered to occur in different time periods. This lends further support to the definition of personality given by Gosling (2008), as consistency across time would also. Thus, it is essential that the definition of a single time period be made clear by each study.

There are a number of conceptual approaches to identifying consistency, either contextually, temporally or both.

1.1.1.1 Contextual consistency

There are two approaches used to analyse behaviour across contexts, either contextual generality or contextual plasticity. Both require behavioural trait scores from two or more individuals in two or more contexts. Contextual generality describes the extent of

the correlation between the behavioural scores of these individuals (individuals 1 and 2 in **Figure 1.1.1***a*). Contextual plasticity is the converse of this, identifying the extent of the variation (plasticity) between individuals (individuals 1 and 2 in **Figure 1.1.1***b*).

1.1.1.2 Temporal consistency

Several approaches are available for analysing temporal consistency. Differential consistency is the same concept as contextual generality, but calculates the correlation of behavioural scores of individuals across different time periods (between individuals 1 and 2 in **Figure 1.1.1***c*). Structural consistency (**Figure 1.1.1***e*) combines both contextual and temporal consistency measurements. This measurement requires that a single time period be long enough to cover a number of contexts. Contextual generality is calculated for a group of individuals between contexts in a number of time periods. Contextual generality scores within each time period are then compared. Structural consistency refers to the extent to which these correlations are preserved across time periods. Mean level consistency (**Figure 1.1.1***d*) combines behavioural scores of individuals into groups. The same group of individuals is then analysed in two or more time periods. The mean behavioural score of the group is compared between these time periods, and the between time period difference in mean values (accounting for within-time period variation) is analysed. This measure takes into account the behaviour of a group within a population and as a result does not directly provide information about individual personalities. What it does provide is an overall indication of the behaviour of the group between time periods, a baseline with which to compare individual behavioural changes. Individual stability (**Figure 1.1.1***f*) is the only of these analyses which considers only one individual. Simply, it is a measurement of the change in an individual's behaviour score along an axis from one time period to another, irrelevant of any other individual's behavioural scores.

Essentially, contextual generality and differential consistency identify CIDs across contexts and time respectively. It is these comparisons which incorporate statistics to measure repeatability levels. These analyses would be suited for use across a number of behavioural traits to identifying personalities in the broader sense, covering only consistency across context or time alone. Structural consistency accounts for both context and time period, and thus is better suited to conservative definitions of personality.

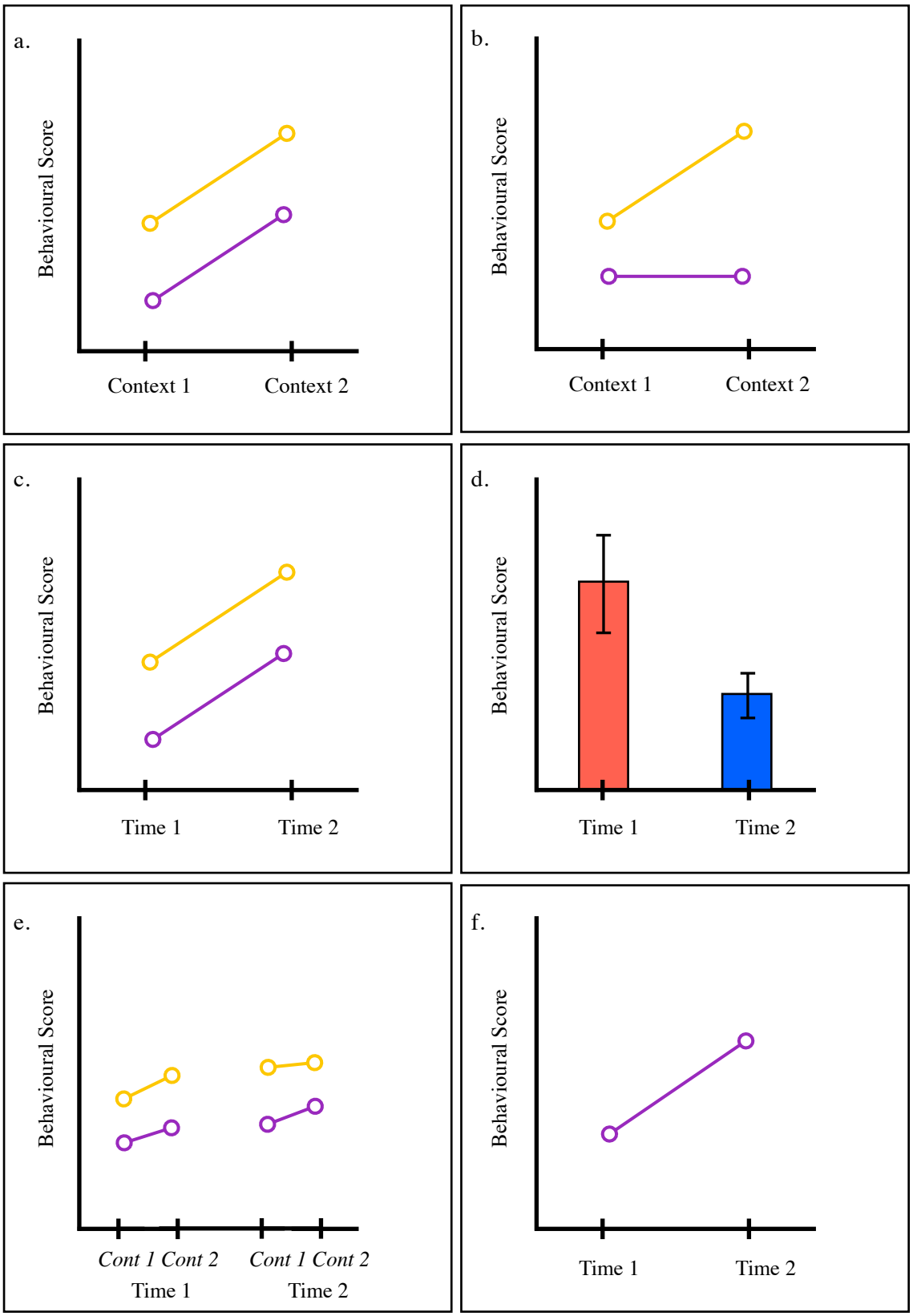


Figure 1.1.1 examples of measures of consistency. a. Contextual Generality; b. Contextual Plasticity; c. Differential Consistency; d. Mean Level Consistency; e. Structural Consistency; f. Individual Stability. In graphs a. to c. and e. to f. individual 1 is represented by the purple line while individual 2 is represented by the yellow. In graph d. consistency is measured between groups of individuals and in this graph, the group at time 1 is represented by the red bar while the group at time 2 is represented by the blue. Adapted from descriptions in Stamps & Groothuis (2010).

1.2 MAINTAINING PERSONALITIES

At the species level, different populations of the same species often evolve adaptive behavioural types to cope with their local environment (Sih *et al*., 2004). For example, funnel web spider (family Hexathelidae) populations are found in regions of plentiful food availability as well as areas with sparse resources. Populations in regions of limited food availability have been found to develop increased levels of aggression in order to capitalise on the resources which are available. The increased aggression levels are not limited to contexts of food acquisition, instead aggression appears higher across all contexts (Sih *et al*., 2004). Changes to a whole population's behaviour in this way are logical, as all individuals are experiencing similar, if not the same, situation (defined in **Table 1.1.1**).

The existence of CIDs are not so easily explained. Individuals within a population must be expressing different levels of behavioural traits, despite all being under the same, or similar, conditions. The classic view of behaviour is that there is no limit to its plasticity. Every individual should be able to express the optimum behaviour for each environment it finds itself in (DeWitt *et al*., 1998; Sih *et al*., 2004). Indeed, superficially this would seem to make the most sense, individuals expressing the optimum behaviours in all situations, and with the ability to vary these behaviours appropriately, should have the highest fitness. However, if CIDs are present in populations, it would seem that this cannot be the case. Behaviours appear to be limited in their plasticity, allowing some individuals to express consistently higher or consistently lower levels of behavioural traits than others, irrespective of the environmental situation they find themselves in (Sih *et al*., 2004). This suggests that some members of the population will be expressing sub-optimal behaviours for certain situations and will therefore be at a disadvantage.

More recent research presents that personality and plasticity need not be mutually exclusive concepts. Sih (2011), for example, adds to his 2004 definition of behavioural syndromes (BS) to say that the presence of these CIDs does not require that the individual express no plasticity. For example, Adriaenssens and Johnsson (2011) describe a situation in which rainbow trout (*Oncorhynchus mykiss*) alter their boldness levels according to life experience. Arguably though, there must be some limitation to plasticity in order for CIDs to exist. Therefore, there must be some function or benefit to this limitation. Again, Adriaenssens and Johnsson (2011) identified a wide variation in exploratory and aggressive behaviours in a single population of brown trout (*Salmo trutta*, L.), suggesting success of a number of life strategies despite a highly selective environment. In the following sections, mechanisms suggested

for behavioural consistency with limited plasticity are reported (section 1.2.1) and a variety of models and empirical experiments concerning the appearance of CIDs in single populations are presented (section 1.2.2).

1.2.1 Potential selective processes underlying time/context consistent behaviours

There have been a number of mechanisms suggested for individuals displaying limited behavioural plasticity. These are reported briefly in this section, and their links to CIDs in single populations are presented in more detail in section 1.2.2.

1.2.1.1 Costs of and limits to plasticity

Despite the obvious benefits of phenotypic plasticity (be it morphological or behavioural), only complete plasticity could ensure that an individual maintained an optimum phenotype- or behaviour-situation match at all times (DeWitt *et al*., 1998). The behavioural environment can change instantaneously, unexpectedly and often without warning (for example, the appearance of conspecifics requiring new social interactions). Although behaviours can be altered instantly, adaptive phenotypic plasticity is often delayed by the flexibility and cost to make alterations to the internal 'machinery' (DeWitt *et al*., 1998). This machinery may be involved in coping with these new behaviours or situations, and thus would limit the benefits of new behaviours for a time. Therefore even behaviourally plastic individuals are likely to spend a period of time with unsuitable mechanisms for interpreting or expressing the correct behaviours for a situation. Similarly, although plasticity can improve reactions to the immediate external environment, benefits will not necessarily extend to surrounding environments. In fact, responses to a change in environment are likely to be impaired as information regarding different environmental states will be reduced while they are not being experienced (Dall *et al*., 2004). It is likely that, although plastic individuals would be more capable of adapting to a range of situations than fixed individuals, the latter should be expected to achieve an overall more successful phenotype-situation match. More succinctly, "A jack of all trades is a masters of none". DeWitt *et al*., (1998) use a botanic example, describing an environment in which long-stemmed plants are favoured. They suggest that, although individuals capable of plasticity would be well prepared for any environment, those plants which grew long stems regardless of environmental information would be able to produce longer stems than the changeable individuals and thus have a greater success in this environment (DeWitt *et al*., 1998). This, too, could be true of behaviours. Those individuals which express a certain level of a behavioural trait regardless of the external environment may be capable of a more appropriate expression of this trait than a plastic individual.

1.2.1.2 State dependence

If adaptive phenotypic plasticity is limited, optimal behaviour may not be directly linked to the external environment. Instead, organisms may assess the optimal behaviour with regard to their internal state (see **Table 1.1.1** for definition of state). This is likely to be the outcome of noisy environmental signals. If signals are unclear, the ability to behave 'plastically' may prove more costly than simply basing responses on the internal state of the individual (McElreath & Strimling, 2006). As previously mentioned, the 'cost' of altering internal machinery linked to the production, understanding of and response to different behaviours can be fairly high, while the speed of altering these mechanisms can be slow (DeWitt *et al*., 1998). Internal states are often more stable than the external environment. Many internal states are impossible or, at best, extremely costly to alter, for example, basal metabolic rate or brain structure (Wolf & Weissing, 2010). Behaviours anchored to these states should, themselves, be stable as long as the state itself is stable. However, internal states can in some instances be labile (vary over time). Behaviours anchored to these variable internal states would themselves be expected to be variable. Behavioural consistency can still occur, however, despite these changeable internal states by way of positive feedback mechanisms, discussed in detail in section 1.2.2.3.

1.2.2 Potential mechanisms for the maintenance of CIDs in a population

1.2.2.1 Frequency dependent selection

Frequency dependent selection has been linked to CIDs for a number of years, since the emergence of evolutionary game theory (Maynard Smith, 1982). Frequency dependent selection can be found in populations with "tactical flexibility" in which individuals adopt different strategies to increase their fitness. When the relative fitness of a strategy is negatively correlated to the proportion of the population employing the strategy, this becomes known as negative frequency dependent selection (Wolf & Weissing, 2010). There are two possible mechanisms by which members of the same population will employ different strategies; either each member spends a defined proportion of time utilising every available strategy, or individuals remain true to one strategy alone. It is the latter mechanism which would be responsible for maintaining long term consistent individual differences (Dall *et al*., 2004).

In the negative frequency dependence model by Wolf *et al*. (2008; reviewed in Dingemanse & Wolf, 2010) individuals were given the 'opportunity' to respond to a constantly changing environment or to be unresponsive to environmental cues, and therefore not incurring an energetic sampling cost. The task set in this model was to forage in an environment in which food patches

were transient. The model found that the responsive behavioural type was limited by negative frequency dependent selection; although they were able to identify the best patches in which to forage, the greater the number of responsive individuals in the population, the lower the benefit per individual. Eventually, the benefits gained from finding these high quality patches became so spread between individuals in the population, they no longer outweighed the costs of scanning the environment (Dingemanse & Wolf, 2010).

A similar model was produced by Barnard & Sibly (1981) to assess the benefit of a minority behavioural trait in foraging situations. In this model individuals were either producers, finding hidden food sources in the environment, or scroungers, taking food which had previously been found by another individual. In this model, benefits enjoyed by the scrounger behavioural type are limited by negative frequency dependent selection. Resources are limited by the efficiency of the producers, a population consisting of mostly producers would be expected to obtain a large number of resources from the environment, this in turn would mean a large volume of resources available to the few scroungers. However, a population with a high proportion of scroungers means fewer available resources (due to a lower number of producers) spread across a greater number of individuals (Wolf & Weissing, 2010). Barnard & Sibly (1981) tested their model using a captive flock of house sparrows (*Passer domesticus* L.). The tests supported the model to a degree: scroungers enjoyed a higher foraging payoff in the company of a number of producers; however, when producers were in far greater numbers than scroungers this no longer held true (Barnard & Sibly, 1981).

Experimental data has not always supported the link between negative frequency dependent selection and the maintenance of personalities. Kurvers *et al.,* (2012) used the producer-scrounger model to test the relationship between boldness in foraging and CIDs in a population of barnacle geese (*Branta leucopsis* Bechstein, 1803). It was expected that shy individuals (the 'scroungers') would benefit from foraging in a group dominated by bold individuals (the 'producers') and vice versa. Instead, it appeared that all individuals, regardless of personal boldness score, enjoyed greater foraging success when forming groups with individuals with a high boldness score. This, in turn, suggested that negative frequency dependent selection was not the driving factor behind the existence of personalities in the population. However, all individuals of this species are known to use the scrounger technique to an extent, they do not remain true to either producer or scrounger behaviours in all situations (Kurvers *et al.,* 2012). According to the description given above (from Dall *et al.,* 2004) this may be an unreliable trait by which to test the link

between negative frequency dependent selection and personality. Another possibility may be that consistency would be better identified within time allocation (i.e. individuals vary consistently with regards to the time they allocate to acting as a producer or a scrounger). Further data on time allocation would be required to assess this possibility.

1.2.2.2 Social benefits of CIDs

Behavioural consistency often brings with it a number of social benefits. For example, in a population in which the members have developed eavesdropping (watching the interactions of others to gain information for future personal interactions) and consistent behaviour, individuals can assess how they should behave in an interaction. An individual which is able to eavesdrop on the interactions of a behaviourally consistant conspecific could better judge the likelihood of winning the interaction itself. This reduces the likelihood that a weaker individual would enter into an aggressive interaction with a stronger conspecific, and risk loss of energy and possible mortality. Similarly, if a strong individual initiates a fight with a weaker individual, the weaker individual would be prepared to take a submissive approach, again avoiding aggression, loss of energy and possible demise (Wolf & Weissing, 2010). This should reduce the overall number of conflicts as eavesdroppers would only enter into a fight they expect to win. Thus, benefits are enjoyed by both members of the interaction.

Furthermore, within a single population, members are likely to have similar resource requirements and a shared optimal niche to suite these needs. This can be in terms of food requirements, territory and other important resources. To reduce conflict, niche differentiation is employed. Some members will alter their niche requirements slightly, or take sub-optimal niches if the most suitable niche is already taken (Bergmüller & Taborsky, 2010). In the long term this method leads to an increased fitness for the displaced individuals, balancing the costs of a sub-optimal niche with the benefits of reduced competition. Behavioural alteration for niche differentiation produces a range of social niches in a single population. For example, in Barnard & Sibly's (1981) model, 'producer' would be one social niche, while 'scrounger' another. Leaving one social niche and joining another may be costly if this incurs increased competition with members of the new social niche. For this reason, the social niche concept is expected to maintain CIDs in a population (Bergmüller & Taborsky, 2010).

1.2.2.3 External interactions with internal state

Stochastic events in an individual's past or different developmental experiences between individuals can alter the internal state of one member of a population with relation to

another. The timing, in terms of the life cycle, at which an experience occurs, may alter its effect and impact on behavioural development (Sih *et al*., 2004). This in turn can affect the observed cost-benefit ratio of various behavioural traits (Dingemanse & Wolf, 2010; Wolf & Weissing, 2010). The difference in developmental experience is likely to lead to individuals holding different assets. Those which have spent time exploring the environment at a young age are more likely to identify suitable grounds for foraging and/or reproduction and therefore enjoy higher assets than those which have not explored as much (Luttbeg & Sih, 2011). The assimilation of assets could affect the way individuals behave across other contexts and through time. Those with high assets may become more cautious, in order to protect these assets, while those with low assets may become more bold as they have less to lose and more to gain with explorative behaviour. This negative feedback mechanism is known as the "asset protection principle" (Clark, 1994; described in Luttbeg & Sih, 2011). However, negative feedback mechanisms act to stabilise states, and as such, over time all individuals should equalise in their assets. Therefore, the "Asset Protection Principle" could not be responsible for long term CIDs (Luttbeg & Sih, 2011). If, conversely, members of the population experienced positive feedback, behavioural traits may become stabilised at different levels dependent on the state of the individual, and as such CIDs may arise (Rands *et al*., 2003; Dingemanse & Wolf, 2010; Luttbeg & Sih, 2010; Wolf & Weissing, 2010). Consider a pair of foraging individuals (modelled by Rands *et al*., 2003). In this model Individual A (from here known as **A**) has lower energy reserves than Individual B (known as **B**). **A** will reach the threshold at which foraging becomes essential before **B**, and will enter the foraging grounds first. In doing so, the costs to **B** of foraging are reduced as foraging in pairs tends to be safer than alone. Both animals will then forage and increase their energy reserves. **B**, having higher initial energy reserves, will reach the threshold for rest (where the costs of foraging outweigh the benefits and so return to a sheltered environment is favoured) **A, B** will cease foraging at this point. This increases the risk of foraging to **A**, as it is doing so alone. Once **A**'s energy reserves increase past the point where foraging is essential, **A** will retire to safety, still with lower energy reserves than **B** (as **A** has not yet reached the threshold for rest). Thus, the cycle will continue in this way, stabilising the internal state differences and, in turn, behaviours anchored to the internal state (Rands *et al*., 2003; Dingemanse & Wolf, 2010). A similar positive feedback was modelled by Luttbeg & Sih (2010) describing the development of different levels of boldness according to the initial state of the individual. In this example, the individual with the higher initial state (e.g. more energy reserves, better condition, greater vigour) will be more successful

during interactions with predators than those individuals with lower initial states. This, in turn, reduces the predation risk for the higher state individual, allowing it to increase in boldness as well as giving more time for foraging to further increase the state. Low state individuals are not able to be so bold or forage so extensively as they will have little success if detected by a predator. For this reason, they maintain a low level of boldness (adapted from Sih, 2011, **Figure 1.2.1**).

The impact of an unpredictable environment on the development of CIDs has been identified experimentally in populations of great tits (*Parus major* L.) linking exploration rates in males and females to yearly survival rates (Dingemanse *et al.*, 2004; Dingemanse & Reale, 2005). In years of high food availability, where competition for resources was relaxed, females with low rates of exploration and males with high rates of exploration enjoyed higher survival. The reverse was true in years with low food availability and high competition. Food availability is dependent on the masting of beech trees, an event which occurs every other year, meaning that the environmental fluctuation is fairly regular and so both extremes of exploration rate are favoured

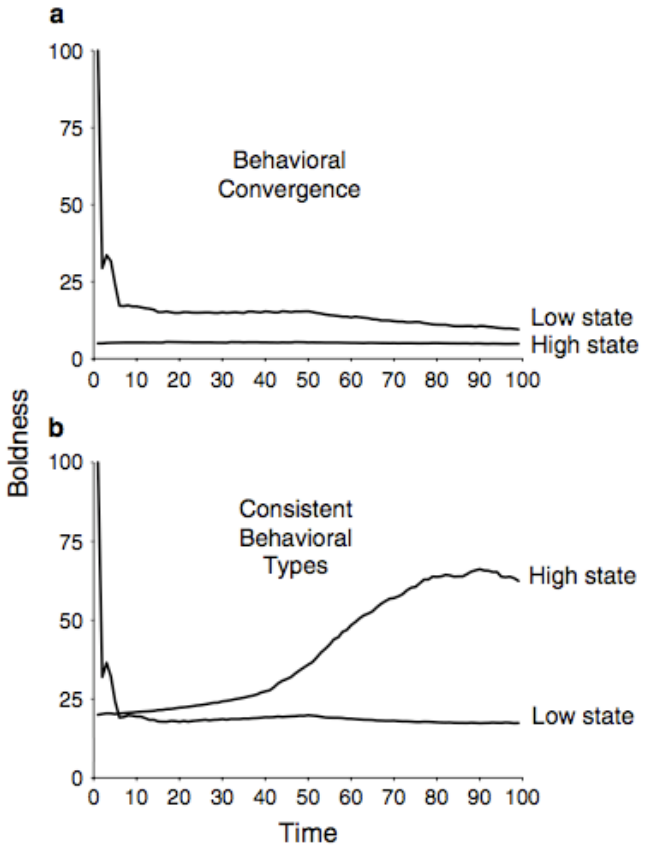


Figure 1.2.1 Comparison between the effects of a negative feedback mechanism (asset protection principle - top) and a positive feedback mechanism (state-dependent safety - bottom) on the boldness score of individuals with differing internal states. Boldness measured on an arbitrary scale; time measured as a series of 'time steps' representing different ecological conditions. Taken from Sih (2011) based on the model produced by Luttbeg & Sih (2010).

equally but not simultaneously (Dingemanse *et al.,* 2004). This is displayed in **Figure 1.2.2**.

1.2.2.4 Internal state (physiology)

The natural variation in the physiology of individuals; for example hormonal balance, metabolic rate, secretion of certain enzymes, will alter the observed cost-benefit of certain behaviours across contexts. Individuals must develop and hone mechanisms (whether these be behavioural or physiological) which respond to external warnings and stimuli, in order to ensure survival. The physical state of the individual will determine how sensitive these mechanisms need to be (McElreath & Strimling, 2006). This links to the "smoke detector principle" described by Nesse (2001 & 2005), which states that the sensitivity of survival mechanisms employed by an organism should be based on the cost-benefit ratio of false alarms. For example, a behavioural mechanism in response to a

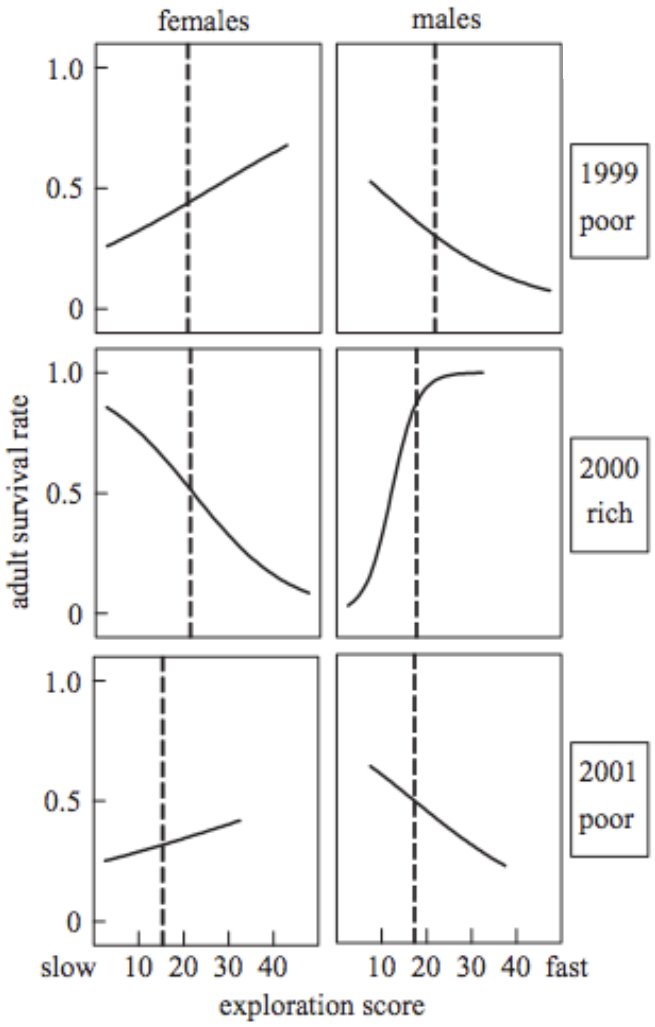


Figure 1.2.2 The relationship between exploration scores (x-axis) of female (left) and male (right) great tits and survival rate (y-axis), during years of poor food availability (top and bottom) and rich food availability (middle). Figure taken from Dingemanse *et al.*, (2004)

predator may be to cease current activities and move to cover. This decreases time for foraging, territory protection and finding a mate. For an individual in a highly predated area, having an extra-sensitive response to potential predator stimuli would ensure cover is reached before being captured. The costs of false alarms in this situation would be minor in comparison to the benefits gained by finding cover in response to any possible predator stimuli. Likewise, an individual with access to good food reserves may not be badly affected by the energetic costs of false alarms, but would still gain benefits from a highly sensitive predator detection mechanism. Conversely, an individual in a well protected area and/or area of low predation rate or food availability, may not benefit from such a sensitive mechanism. In these situations, a highly sensitive mechanism would cause predator-avoidance responses in a number of situations where they were not required (i.e. a false alarm). This would increase energy consumption in order to find cover, and reduce time for foraging. In this situation, predator detection mechanisms are likely to be less sensitive (Nesse, 2001 & 2005; McElreath & Strimling, 2006). This in itself is unlikely to lead to consistent individual differences; all members of a population will be experiencing the same, or similar, environmental cues and so it would be expected that response mechanisms would have the same sensitivity throughout the population. However, an individual with more experience around the territory may have prior knowledge of short cuts to a safer area and would therefore benefit from a less sensitive predator-avoidance mechanism than a conspecific with less experience. Similarly, differences in individual development resulting in increased movement capabilities would require less sensitivity to predators. In this way, early life experiences could affect inbuilt response mechanisms and as such produce consistent differences between individuals in terms of behavioural responses (McElreath & Strimling, 2006).

1.2.2.5 Between- and within-generational bet-hedging

Uncertain environmental conditions can trigger a process known as bet-hedging. This term was originally used to describe a plant's ability to 'decide' whether to produce a bulk crop of seeds in a year or whether to spread the production of seeds over a number of years. This distinction between different life history strategies is a result of uncertainty of environment (King & Masel, 2007). In behavioural ecology there are two types of bet-hedging, between- and within-generational. Between generation bet-heading describes a single genotype producing offspring with a number of different phenotypes or behavioural strategies, in order to account for environmental uncertainty and increase the fitness of their genotype (a 'risk-spreading' strategy, Hopper *et al.,* 2003; Wolf & Weissing, 2010). This may be used either in

situations where the environment changes constantly, or in the case of noisy environmental signals (Reddon, 2012). It is so called because the success of an individual's 'bet hedging' attempts must be considered across a multitude of generations. Initially, an individual producing the greatest number of differing offspring phenotypes would appear to have the greatest fitness as there is a higher chance of a successful phenotype being propagated. However, when considered across a number of generations, it is infact the line of individuals best able to match offspring phenotype to the environment that will have the highest survival success (Hopper *et al*., 2003).

The second form of bet-hedging is within-generational bet-hedging. Within generation bet-hedging describes the spatial or temporal separation of offspring rather than an alteration of phenotype, to reduce the risk of stochastic events (e.g. predation, nest destruction) destroying all progeny (Hopper *et al*., 2003). In this case, the fitness of an individual can be increased simply by increasing the number of offspring produced and spreading them over a number of nests, for example. As identified by Hopper *et al*. (2003) there is widespread confusion regarding the distinction between these two types of bet-hedging. It is the former of these (between generation bet-hedging) which would directly produce CIDs within a population (although not all phenotypes may survive) and links to the environmental impacts described in section 1.2.2.3. For example, the great tits described by Dingemanse *et al*., (2004) may produce offspring of varying exploratory rates in order to account for the yearly change in food availability. This would ensure that, regardless of this food availability, there would be an increased chance of a phenotype being propagated which would survive the season and continue the genotype.

1.2.3 Consistent individual differences in context

Section 1.2 has reported a variety of potential explanations and models for the development and maintenance of CIDs despite the potential negative fitness impacts of reduced behavioural plasticity. Of these, internal state and social interactions can be affected by early life experiences. For this reason, in species which show parental care, the behaviour (and as such personality) of the parents may have an impact on the future behaviour of the offspring. In particular, as the timing of experiences in an individual's life may dictate behavioural development (Sih *et al*., 2004), the personality and consequent actions of their parents may have an effect on this development. In order to fully understand personality development, it is therefore important to identify and understand the presence of individual variation in parenting styles, and ways in which this may alter both the behaviour and physiology of the infant.

The following subchapter (1.3) deals with empirical evidence for both the existence of CIDs in parental behaviour and for effects of these CIDs on offspring.

1.3 DEFINING MOTHERING STYLES

So far, this thesis has discussed the definition of non-human personalities, and brought together current model evidence to account for the maintenance of these consistent individual differences (CIDs) in a single population. This section will consider an aspect of these CIDs relating maternal behaviour; otherwise known as maternal or mothering styles (Hill *et al*., 2007). Mothering styles are defined as "the occurrence of consistency over a number of periods of maternal care with regard to relative differences between mothers for parameters of maternal behaviour" (Albers *et al*., 1999). In short, they are CIDs in maternal behaviours of a population across a number of rearing periods. Not all studies have used a definition which takes into account a number of rearing periods. For example, when studying dolphins (family Delphinidae; an animal which cares for young for up to four years), Hill *et al*. (2007) looked for consistency in maternal behaviour across the first year of the calves' life. Likewise, in a study of vervet monkeys (*Chlorocebus pygerythrus* Cuvier, 1821) Lee (1984) used two samples per mother-infant pair for the first 12 weeks of the infant's life, but with no repeat between breeding seasons. The definition given by Hill *et al*. *(*2007); "The extent to which a mother nurtures, protects and allows her young to explore their world", though vague, gives a good overall description of mothering styles without necessarily including several rearing periods. Mothering styles have been identified in a range of species, from non-human primates such as vervet monkeys, rhesus and Japanese macaques (*Macaca mulatta*, Zimmermann 1780; *Macaca fuscata*, Blyth 1875 respectively) and brown capuchin monkeys (*Cebus apella*, L.) to Guinea-pigs (*Cavia porcellus*, L.), various rodents, the family Delphinidae and humans (*Homo sapiens*, L.) (Lee, 1984; Albers *et al*., 1999; Francis *et al*., 1999; Meaney, 2001; Bardi & Huffman, 2002; Weaver & de Waal, 2002; Champagne *et al*., 2007; Hill *et al*., 2007; Maestripieri *et al*., 2009).

A number of studies conclude that mothering styles are most prominently expressed in affiliative and rejective behaviour (Lee, 1984; Albers *et al*., 1999; Maestripieri *et al*., 2009). Indeed, in human mothers the only consistent mother-offspring interaction was found to be affection (from Dunn, 1975). By qualifying affiliative and rejective behaviours, Bardi & Huffman (2002) produced three distinct groups for mothering styles:

- Rejective mothers: regularly interrupt contact with their offspring; prevent infant-initiated contact; extended periods of time spent out of contact with the offspring.

- Protective mothers: maintain contact with the infant; restrain straying infants; maintain visual contact with

the infant when physical contact is broken.

- Laissez-faire mothers: tolerate infant-initiated contact; do not restrain straying infants.

Although it is likely that all mothers will show aspects of both protective and rejective behaviours, it is the extent to which these traits are expressed which define the maternal style of an individual (Hill *et al*., 2007).

1.4 THE IMPORTANCE OF MOTHERING STYLES

Early life interactions with parents (be they biological parents or otherwise) are known to have a profound effect on offspring behaviour in a number of species. Indeed, the matter of behavioural transmission or heritability of certain behavioural traits has become of such importance in the past two decades that there has been a movement of research into the field of psychology, in order to determine the effects of early family environments on the growth and well-being of children (Bardi & Huffman, 2006). A collection of studies (summarised in Caldji *et al*., 2000 and Meaney, 2001) have linked neglect and low level abuse experienced in the home environment at a young age, such as strict physical discipline, with serious negative effects on mental well-being later in life, increasing the risk of illnesses such as depression. Similarly, a poor emotional relationship with parental figures can increase the likelihood of poor physical health as an adult (Caldji *et al*., 2000; Meaney, 2001). In order to begin assessing the impacts of different mothering styles on offspring behaviour in non-human species, it is first essential that CIDs be identified in various maternal behaviours.

1.4.1 Empirical evidence for mothering styles

Maternal behaviour in Guinea-pigs is well studied. Guinea-pigs are capable of short time periods between births, and will accept and care for young which are not their own, making them ideal test subjects for the consistency of mothering styles (Albers *et al*, 1999). This study used repeated observations on ten test subjects across four consecutive litters. Cross fostered pups were used in two of these four litters for each individual mother to ensure that maternal behaviour was not influenced by the behaviour of her own pups. Using focal videos, the uninterrupted behaviour of the mother was observed for 90 minute intervals. Behaviours were sampled continuously, using predetermined maternal behaviour categories. Albers *et al*., (1999) found that affiliative and agonistic behaviours between mother and offspring were ideal for characterising individual mothering styles in *C. porcellus* , as these behaviours were found to be repeatable. Repeatability extended across all four litters, including those using cross fostered pups. In addition to this, the locomotive behaviours of the mother and time spent apart from pups was found to be repeatable between litters. Other, more species specific behaviours, have shown evidence of consistency and as such can be classed as mothering styles. For example, dolphin mothers are known to discipline their calves to discourage dangerous behaviour; time spent disciplining young has been found to vary consistently between individuals within the first year of the offspring's life (Hill *et al*., 2007).

In species with bi-parental care, generally only one parent displays repeatability between broods; the other parent is more reactive to the specific situation. In pairs of wild *P. domesticus,* males show repeatable food provisioning behaviour both within and between broods (Schwagmeyer & Mock, 2003; Nakagawa *et al*., 2007). Schwagmeyer & Mock (2003) used artificial handicapping of both male and female birds independently, slowing their rate of food provisioning. Male food provisioning rates remained the same, regardless of that of the female, suggesting that males take a more pro-active response to food provisioning. Female food provisioning rates fluctuated depending on that of the male; females in a pair with a handicapped male increased their rate of food provisioning to account for this. This suggests that females are more responsive to changes in situation, and as such take a reactive response to food provisioning (Schwagmeyer & Mock, 2003). Nakagawa *et al*. (2007) obtained video observations of nesting *P. domesticus* over a number of breeding periods, during which some male-female partnerships retained the same individuals, and others changed. Again, male food provisioning rates were found to be repeatable while female rates altered depending on that of their partner. This did not differ between those individuals with the same partner from previous years to those with new partners (Nakagawa *et al*., 2007). Both papers conclude that male repeatability may be a method of attracting females. Those which show high food provisioning rates in one year can be assumed to do so in the future. This allows sexual selection by females. Female responsiveness compensates for any changes in behaviour or shortfalls by their partner (Schwagmeyer & Mock, 2003; Nakagawa *et al*., 2007). Pied flycatchers (*Ficedula hypoleuca*, Pallas 1764) also provide bi-parental care to their offspring. In this case, however, females show consistent levels of expenditure while males do not. It is thought that this may be a result of lower confidence of paternity on the part of the male. Extra-pair copulation is common in *F. hypoleuca* and as such paternity cannot be assured (Potti *et al*., 1999).

In non-human primates, mothering styles have often been linked to those observed in humans and techniques used to analyse mother-offspring (MO) relationships have been transferred from psychology to animal behaviour. For example Weaver and de Waal (2002) use Attachment Theory (a study of how secure a human infant feels in the relationship with its mother) in order to compare mother-offspring relationship quality (MORQ) amongst brown capuchins. By obtaining focal observations of uninterrupted MO behaviour, the ratio of affiliative to agonistic behaviours of each pair could be calculated, relative to that of all other MO pairs at the same developmental stage. This allowed a non-invasive, quantitative measure of MORQ for each pair which could be compared amongst the group.

MORQ measures were found to be repeatable across developmental stages (Weaver & de Waal, 2002). These protective or rejective tendencies have been linked to the physiology of the individual mothers. For example, Maestripieri *et al*. (2009) gave each mother in their study group of free ranging *M. mulatta* a maternal rejective index (based on the frequency of rejective and agonistic interactions between mother and offspring during the focal period) and compared this score to blood plasma cortisol levels of the mother. A positive correlation was found between the frequency of rejective behaviours performed by the mother and the level of cortisol found in the plasma (Maestripieri *et al*., 2009). The same study suggests that the maternal style of an individual is developed from, or influenced by, the maternal style of her mother. It is important to understand these aspects of MO relationships, as there is growing evidence that maternal behaviour affects aspects of offspring physiological and behavioural development, often in ways which may extend to later life. It is, therefore, essential to our understanding of adult behaviour and personality development, to identify the potential impacts of parental personalities on their young.

1.4.2 Empirical evidence for the impact of mothering styles on offspring behaviour

Various breeds of rat (genus *Rattus*) have been used to examine the effects of maternal behaviour on the future behaviour of offspring. Licking and Grooming (LG) is a behaviour carried out by mothers of all breeds and often occurs in conjunction with nursing. CIDs have been identified in the duration of this LG behaviour, and have been found to be repeatable not just within, but between litters (Francis *et al*., 1999; Caldji *et al*., 2000). Mothers can be categorised according to the period of time they spend performing this LG behaviour, as either high LG (spending long periods of time licking and grooming their offspring) or low LG (spend only a small amount of time on this behaviour). Pups of high LG mothers have been shown to cope better with novel situations, with higher rates of exploration and lower response to stress than have pups of low LG mothers. Daughters appear to reflect their mothers, with those from high LG mothers displaying high LG with their own offspring, and those from low LG mothers performing low LG behaviour with their litters (Francis *et al*., 1999; Caldji *et al*., 2000; Champagne & Meaney, 2007). This is known as nongenomic behaviour transmission or behavioural transmission of traits (Champagne *et al*., 2007) as it is not a genetically heritable trait. Cross fostering experiments, transferring rat pups from low LG mothers to high LG mothers, found the behaviour of the offspring to be determined by the behaviour of the adoptive mother and not the biological mother. This suggests that it is the behaviour of the maternal figure which influences the development of offspring behaviour (Francis *et al*., 1999;

Caldji *et al.,* 2000; Champagne & Meaney, 2007). These studies found that the process of the mother licking and grooming the pups influenced the development of stress-responsive neuropeptide receptors, namely glucocorticoid receptors, in the pups' hypothalamus (Francis *et al.,* 1999; Meaney, 2001; Weaver *et al*., 2004). It is these receptors which, in turn, determine the level of LG behaviour displayed by the pup on becoming an adult (Champagne & Meaney, 2007). Again, cross fostering experiments have shown that the level of these neuropeptide receptors in the hypothalamus is a result of maternal behaviour and is not hereditary (Weaver *et al*., 2004). Interestingly, this development is also dependent on the post-weaning environment experienced by the pups. Champagne & Meaney (2007) exposed both pups from high LG and low LG backgrounds to either standard, impoverished or enriched environments after they had been weaned from their mothers. Environmental enrichment was found to increase the predicted LG levels for rats with low LG mothers, when caring for their own litter. Conversely, impoverished environments were found to decrease the predicted LG levels for rats with high LG backgrounds, instead showing low LG behaviour with their own pups. The same pattern was found with exploratory behaviour of female rats (Champagne & Meaney, 2007). For captive rats, post-weaning handling by humans can act to reverse the effects of low LG experienced as a pup, instead producing an adult with typical high LG behaviour (Francis *et al*., 1999).

Although not all studies have shown a link between maternal styles and offspring behaviour, it can be the case that the wrong maternal behaviour is being used for comparison, or even the wrong measure of this behaviour. For example, as discussed in the previous section (1.4.1), Albers *et al*. (1999) identified consistent individual differences in the affiliative and agonistic behaviours female Guinea-pigs expressed in response to their pups. They also identified consistency in the locomotive behaviour of these mothers (Albers *et al.,* 1999). As a continuation of this study, Albers *et al*. (2000*a*) attempted to link maternal locomotive behaviour to the later exploratory behaviour of their pups. In this paper, they began by scoring mothers according to the amount of time they spent moving and exploring their environment. They then exposed the pups to a novel environment and scored them according to the amount of time spent performing exploratory behaviours such as sniffing at the environment, and time spent away from the safety of the start position. In this case, no link was found between the exploratory behaviour of the mother and that of her pups (Albers *et al*., 2000*a*). However, by altering the measurement of maternal behaviour used in the experiment, Albers *et al*. *(*2000*b*) were able to observe a correlation between pup

exploratory behaviour and the duration and frequency of separation bouts between mother and pups. Mothers and pups were placed into one of three groups: A, B or C. For those in group A, the mother was separated from the nest three times a day, each time for three hours. In group B, mothers were removed from the nest six times a day, three of which lasted for two hours, and three lasting one hour. The final group, group C, experienced nine separations per day each for only one hour. The pups were, again, placed into the novel environment test and scored for their exploratory behaviours. Pups from group A were found to explore more than pups from groups B or C. They spent more time walking, more time away from the start position and more time sniffing their surroundings. These pups, though experiencing the lowest frequency of separations per day, endured long periods of time with no maternal contact. This suggests that extended periods of time without maternal input actually acts to increase boldness and environmental investigation in the young (Albers *et al*., 2000*b*). The same pattern was observed by Bardi & Huffman (2006) in both *M. fuscata* and *M. mulatta*. There appeared to be a positive correlation between the level of maternal rejection behaviour (in which the mother rejects infant-initiated contact) and the enterprising behaviour of her young (Bardi & Huffman, 2006). A link between increased calf independence and decreased maternal control has also been reported in dolphins (Hill *et al*., 2007).

Linked to the apparent negative effects of over-protective mothering are findings by Maestripieri *et al*., (2009) discussed briefly at the end of section 1.4.1. This study on free ranging *M. mulatta* identified not only the physiological basis of maternal behaviour, but also impacts of maternal behaviour on the offspring. Measurements of blood plasma cortisol (a steroid hormone, released in response to stress) in the offspring were compared against the mother's protective or rejective score. Results suggested that maternal scores of protectiveness were positively correlated with blood plasma cortisol levels in the infant. As such, these infants were expected to be more sensitive to stress in their adult life. Blood cortisol levels were not transmitted genetically from mother to offspring; cortisol levels in the mothers' blood plasma positively correlated with their rejective score but had no link to their protective score. Previous studies using cross fostered infants showed that blood plasma cortisol levels were an example of nongenomic transmission. Not only did maternal behaviour impact upon the physiology of the infant but also the behaviour. Infants of mothers with high protective scores showed increased contact breaking, whereas those with high rejective scores showed increased contact initiation (Maestripieri *et al*., 2009). Interestingly, for human children, the more accessible, relaxed

and tender a mother, the more secure the bond between mother and infant. A procedure known as the 'strange situation' was used to assess relationship security between mother and infant. Infants were separated from their mothers and placed in a novel environment with an unknown adult. Those with tender mothers were seen to be distressed by this separation, but quickly reassured by the return of their mother. Those with less available, more stressed mothers were not so easily consoled by the return of their mother and continued crying, or were entirely unphased by the situation, acting as though nothing had changed (Weaver & de Waal, 2002).

In social species, short bouts of separation can alter the development of an infant. For example, in *M. fuscata*, higher levels of maternal rejection were found to lead to increased contact and sociability with the group by the infant (Bardi & Huffman, 2002). In capuchin monkeys, mother-offspring relationships higher in affiliation than agonism are classed as secure. Infants in secure relationships with their mother reacted to infrequent rejective or aggressive behaviour by the mother with an affiliative response. This suggested that the infant was not intimidated by the mother and that affiliative interactions lead to greater infant confidence (Weaver & de Waal, 2002).

Wild aquatic and semi-aquatic mammals have received less attention in terms of personality and mothering styles research than land mammals. Though many mentions have been made during section 1.3 to research on dolphins, comparatively there is a far greater volume of research available on non-aquatic species. This is presumably because behaviour is far more difficult to follow and assess once the focal individual enters the marine environment. Pinnipeds therefore provide an excellent opportunity for researching mothering styles, as all members give birth on shore. This study will build upon existing knowledge of pinniped behaviour in an attempt to obtain preliminary evidence for mothering styles in the grey seal *(Halichoerus grypus* Fabricius, 1791).

2. BACKGROUND AND STUDY RATIONALE

2.1 THE GREY SEAL (*Halichoerus grypus)*

The grey seal (*Halichoerus grypus*) is a marine mammal of the family phocidae, found in the North Atlantic Ocean, North Sea, Barents Sea and Baltic Sea (Thompson & Duck, 2010). In 2010, it was estimated that the UK alone accounted for 45% of the total grey seal pup production (Thompson & Duck, 2010). This is predicted to rise, given the general increase in size of individual breeding colonies around the UK between 1950 and 2010 (Thompson & Duck, 2010). Breeding colonies can be found all around Britain, including the South West of the UK and Wales, however 90% of all breeding individuals are located in the north of Britain, in particular in Scotland (Thompson & Duck, 2010). These populations can be found on a variety of surface terrain, such as open beaches with greater ease of access to the sea, and offshore islands often with rough terrain and limited entry points into the sea (Redman *et al*., 2001). In the UK, pupping (the process of giving birth to a pup) takes place in autumn, however the exact time depends on the location of the colony. In the South West of the UK, the pupping season falls mainly between August and September. The North West sees most of the grey seal pups born between September and November whilst, in the East of the UK, pupping does not begin until late October and can last into late December (Thompson & Duck, 2010).

2.1.1 Behaviour on breeding colonies

Grey seals are polygynous pinnipeds, hauling out onto land and forming mixed sex colonies in which the females give birth to a single pup, care for the new pup, and mate with colony males (Kovacs, 1987; Pomeroy *et al*., 2000*a*). For the duration of their stay on the breeding colony, females fast, surviving and rearing pups using only the resources stored as blubber prior to hauling out (Kovacs, 1987; Redman *et al*., 2001). Females spend the first few days ashore selecting a suitable pupping site (Pomeroy *et al*., 1994). They tend to be loyal to sites from previous years (Coulson & Hickling, 1964), with site fidelity as accurate as to within tens of meters from year to year depending on the topography of the site; for example, the uneven topography at the Isle of May saw a site fidelity radius of around 27m (Pomeroy *et al*., 2000*a*.). Each pregnant female will give birth to a single pup, with which a bond is formed quickly using smell (Coulson & Hickling, 1964; Fogden, 1971). The mother then uses the smell of the pup, and to an extent, pup vocalisations to identify their pup within the colony (Fogden, 1971; Caudron *et al*., 1998; Insley *et al*., 2003). In most

instances, a female will nurse only this pup for the rearing period, with the exception of occasional misidentification or disturbance to the colony leading to allo-suckling (Fogden, 1971; Insley *et al*., 2003). Males do not participate in the rearing of the pups (Smiseth & Lorentsen, 1995a) making maternal behaviour all the more important. Being a species with a low rate of offspring production, quality of offspring must be high (Gray *et al*., 2005). Maternal care usually lasts between two to three weeks, after which the pup is weaned and no more maternal attendance is received. At this point, the female usually mates with one or more males on the colony before returning to the sea (Coulson & Hickling, 1964; Kovacs, 1987; Thompson & Duck, 2010). Pups remain on the colony for a period post-weaning. The length of time for which pups remain on the colony varies depending on the location, for example, in Pembrokeshire pups enter the sea at three weeks of age, whilst on the Farne Islands some have been recorded to stay onshore as long as 40 days (Coulson & Hickling, 1964).

Bull grey seals position themselves within groups of females on the breeding colony and will use aggressive behaviour (from visual threats to violent interactions) to deter other males approaching these females (Twiss & Franklin, 2010). The breeding success of males has been found to be linked to their spatio-temporal positioning within aggregations of females on breeding colonies, and their ability to ward off competition (Twiss *et al*., 2006). Males also fast whilst on the breeding colony. Time must be partitioned between sexual interactions to increase breeding success, aggression in order to maintain position in the colony and resting to conserve stored resources (Anderson & Fedak, 1985). Careful resource allocation can maximise the time spent on the colony, which in turn has been shown to increase breeding success (Anderson & Fedak, 1985).

2.1.2 Current evidence for behavioural consistency in grey seals

There is already evidence for the existence of CIDs among various behaviours in grey seals. These have been found in both cows and bulls. Twiss & Franklin (2010) began by observing bull behaviour across a number of breeding seasons, producing activity budgets for each individual. Highly repeatable individual behaviour patterns were reported, in particular with respect to alert behaviours (Twiss & Franklin, 2010). This was closely followed by the assessment of behaviours displayed by *H. grypus* in response to artificial auditory stimuli (Twiss *et al*., 2011). In this test, response behaviours were monitored across a group of individually identifiable seals located on a breeding colony on the island of North Rona. During the experiment, a remote control vehicle (RCV) was positioned approximately three meters from an individual and an unfamiliar animal call was played three separate times.

After a period of four to fourteen days, this procedure was repeated with the same set of individuals. These individuals consisted of both sexes, though cows with pups were found to be better suited for the test. These cows tended to remain in close proximity to their pup, and therefore to the RCV, ensuring their presence for all three playbacks of the audio call. Results from this test, suggested significant repeatability in response behaviours both in cows and bulls (Twiss *et al*., 2011). Of particular relevance to this study is the high repeatability found in pup-checking behaviour by individual cows. Pup-checking is a behaviour in which the mother raises her head (if on the ground) or turns her neck, and makes a direct and purposeful look at her pup (Kovacs, 1987; Twiss *et al*., 2011). Using the ICC to compare the number of pup-checks made during the first and second test, the study identified a low level of inter-individual variation but a high level of intra-individual variation. This suggested the presence of CIDs (Twiss *et al*., 2011). The same set of females were assessed during periods of no disturbance (relaxed focal; Twiss *et al*., 2012). Relaxed pup-checking rates were calculated from 30 minute focal videos, which were only taken when the female was not being disturbed by other members of the colony. CIDs were also identified in relaxed pup-checking rates (Twiss *et al*., 2012). Interestingly, no CIDs in pup-check rates were found when comparing the same females between disturbed and relaxed situations, suggesting that disturbance affects individuals differently (Twiss *et al*., 2012).

2.1.3 Maternal behaviour and the factors affecting it

Whilst on land, pups face a number of threats. Studies compiling data from several breeding colonies reported that (not including stillbirths and birth-related complications) the main causes of pup death are starvation, injuries from other adults and wound infection (Coulson & Hickling, 1964; Redman *et al*., 2001). Starvation can occur in two forms. The first is through permanent separation of the mother and pup due to disturbance or long distance locomotion by the mother (Pomeroy *et al*., 1994). Starvation can also be the result of the mother failing to supply sufficient milk in order for the pup to form a layer of blubber (Coulson & Hickling, 1964). Injuries and wounds caused to pups are often the result of other protective mothers behaving aggressively towards pups which are not their own in order to protect their offspring (Coulson & Hickling, 1964; Redman *et al*., 2001). Injuries can be caused during male-male territorial aggression, in which nearby pups may get crushed (Coulson & Hickling, 1964). Another cause of injury may be disturbance within the colony, leading to a mass movement of adults without regard for surrounding pups, during which pups are often crushed (Coulson & Hickling, 1964). This means that, in order to ensure pup survival, mothers are required to expend a

portion of their own, limited resources on maintaining a healthy pup. There appear to be three levels of maternal attendance afforded to pups during the rearing period:

- *Nourishment*. This is essential to the survival of pups, and those which are separated from their mother or are not supplied with sufficient milk by the mother do not survive to weaning age (Pomeroy *et al*., 1994; Pomeroy *et al*., 1999)

- *Vigilance and Protection*. This is not always essential to the survival of the pup as the occurrence of aggressive outbursts or disturbance movements are not guaranteed. However, vigilance and protective behaviours may prevent these from causing damage to the pup if they do occur (Coulson & Hickling, 1964; Redman *et al*., 2001).

- *Mother-Pup interaction*. This includes any social interaction (both positive and negative) between the mother and pup. The benefit of mother-pup social interactions in grey seals is not yet known, but it does not appear to be essential to the immediate survival of the pup (Bekoff, 1972; Kovacs, 1987; Twiss *et al*., 2000).

The level of maternal input afforded to pups appears to fluctuate depending on external factors. A number of factors have been suggested to account for these variations and these are discussed in the following subsections.

2.1.3.1 Environmental factors

Female grey seals rearing pups on breeding colonies have often been observed travelling away from their pup in order to gain access to the sea or isolated pools of water (Twiss *et al*., 2000). Remaining in pools or the sea when not nursing their pup allows mothers a method of thermoregulation (Redman *et al*., 2001), a source of freshwater intake (only from pools) (Twiss *et al*., 2000; Redman *et al*., 2001) and potential protection from human predation (Fogden, 1971). Mothers may either rest onshore with their pups for extended periods, or remain in the water, leaving only to nurse the pup. This choice appears to be determined in part at least by the terrain, colony size and ease of access to water. (Fogden 1971; Kastelein *et al*., 1991; Smiseth & Lorentsen, 1995*b*; Twiss *et al*., 2000). Accessibility of the sea is fairly constant over time for any one colony and maternal attendance behaviour can be predicted from this. In broad terms, seals breeding in colonies on flat beaches should find locomotion and access to the sea less costly than those breeding on inland sites with rocky terrain and steep sea entrance points. For this reason, seals on offshore island colonies with steep access points tend to have to make longer journeys in order to access the sea, and will remain in the water for longer to minimise locomotion costs. This reduces the time available to spend with

their pup (Twiss *et al*., 2000; Redman *et al*., 2001). Throughout the breeding season, availability of pools of water may fluctuate depending on rainfall, changing tides and storms (Twiss *et al*., 2000). Temporal variation in pool availability will alter commuting needs of females and formation of a nearby pool may provide easier access to water than the sea (Twiss *et al*., 2000; Redman *et al*., 2001). The tendency to enter the water is not just a trait seen in wild individuals, suggesting that protection from predation is not the only driving force behind this behaviour. A similar preference for spending time away from the pup and in a body of water was observed in a captive grey seal mother-pup pair at the Harderwijk Marine Mammal Park (Kastelein *et al*., 1991). The same female was observed over two rearing seasons, the first without access to the pool and the second with access. When access to the pool was available, the mother spent very little time on land, outside of nursing with the pup. This suggests a preference toward being in the water over being on land by the mother (Kastelein *et al*., 1991). Spatial distribution of seals on the breeding colony appears to have some effect on the time mothers spend ashore with pups. Observations on individual islands within the Farne Islands found that the length of time mothers spent ashore in close proximity to their pup was different for different islands. On the two most crowded islands, North Wamses and Staple, mothers were observed to spend more time ashore with their pups than the less crowded islands, South Wamses and Brownsman (Coulson & Hickling, 1964). It is unlikely that the difference in this instance was due to differential accessibility to the sea caused by the terrain, as North Wamses had easy access to the sea via beaches around its circumference while Staple island was surrounded by high cliffs with limited sea access points. It was therefore concluded that density and size of the colony played a part in determining the extent of maternal attendance (Coulson & Hickling, 1964).

Environmental disturbance, in particular as a result of humans, has come under increasing scrutiny with the increase of tourism to breeding beaches. The presence of humans has been linked to a reduction in the use of potential or current breeding sites by southern fur seals (*Arctocephalus australis,* Zimmerman 1783). Stevens & Boness (2003) identified 70 potential breeding sites across Peru and categorised these as currently used, abandoned or unused. The sites currently used were negatively correlated with human disturbance. Humans are perceived as a threat and cause increased vigilance behaviours as a result of their presence. Currently used breeding sites were positively correlated with the availability of shade and pools, used for thermoregulation. However, it is often the presence of stacked rocks which provide protection from human disturbance by reducing the ease of tourist access, whilst also providing pools and

shade. These structures can be unstable and steep, increasing pup mortality rates. Stevens & Boness (2003) suggest that a trade-off should be made by larger colonies for whom safety in numbers reduces the need for extra vigilance behaviours in response to human presence. Larger colonies will haul out on areas exposed to human disturbance if these provide safer grounds for the pup (Stevens & Boness, 2003). Engelhard *et al*. (2001) compared the weaning mass of pups on two colonies of southern elephant seals (*Mirounga leonina*, L.). The colonies were located on Macquarie Island, both hauling out onto land of similar topography and ease of access to the sea. One colony was accessible by tourists, whilst the other was located on a remote beach with no human disturbance. Pups of weaning age were significantly heavier on the remote colony than those on the disturbed colony. Weaning mass is positively correlated to survivorship in southern elephant seal pups and as such, human presence could be implicated in lower pup survival rates. However, mothers on the remote colony were also longer and heavier than those on the disturbed colony and the ratio of mother to pup mass was approximately equal between sites. This study was therefore unable to conclude that human disturbance was negatively affecting pup growth (Engelhard *et al*., 2001).

2.1.3.2 State dependent factors

During the breeding season, female grey seals fast (Kovacs, 1987; Pomeroy *et al*., 1999), meaning that energetic resources are limited to the blubber they have built up prior to hauling out onto land. As mentioned in the previous section (2.1.3.1), water is a limiting resource for females breeding on colonies with limited access to the sea or pools (Redman *et al*., 2001). Though hydration is generally obtained through internal production of water as a result of the metabolism of fat, grey seals have been seen to favour pupping sites with access to water. Empirical evidence suggests that, whilst on land, grey seals use behavioural thermoregulation to alleviate thermal stress (Twiss *et al*., 2002). Once a pupping site has been selected, a female grey seal must balance the energetic costs of finding water, as well as the risk of leaving her pup alone on the colony, with the benefits of thermoregulation and potentially of re-hydration (Redman *et al*., 2001). Time budgets taken of grey seal mothers on land show extensive periods of rest and inactivity, thought to be a method of conserving both energy and water loss (Harwood, 1976, reported in Smiseth & Lorentsen, 1995*b*). Indeed, while resting, grey seals employ a different breathing mechanism, involving gasping air in short bursts, known as apneustic breathing. This type of breathing lowers the respiration rate, minimising heat production and reducing loss of water vapour in the breath (Redman *et al*., 2001). However, it is essential that each mother partitions her resources between her own survival and that of her pup. This type of

resource allocation has been studied in the southern elephant seal, another species that undergoes fasting during the breeding season (Arnbom *et al*., 1997). By comparing maternal expenditure (measured as mass loss to the mother) of mothers with single pups and mothers nursing twins, Arnbom *et al*. (1997) determined that the mothers most likely had control over this resource allocation. Females with twins did not endure a larger decrease in body mass than those with a single pup. This suggests that females are not willing to risk their survival or future reproductive success by expending excessive resources in order to support their pups (Arnbom *et al*., 1997). This is supported by Pomeroy *et al*. (1999), who state that expenditure by a grey seal mother in one breeding season impacts upon her fitness in the subsequent year. This is discussed in more detail in section 2.1.3.4.

2.1.3.3 Pup gender

This is a much debated factor across all pinniped species, as there is evidence both in favour and against the gender of the pup affecting maternal expenditure. Grey seals are sexually dimorphic and, as adults, males are generally far larger than females. Males must compete during the breeding season to maintain access to females with which to breed (Anderson & Fedak, 1985). For this reason, there tends to be a greater variation in adult male fitness than female fitness. Mothers with male pups are expected, therefore, to invest more into rearing than those with female pups as size has less of an influence on female reproductive success (Kovacs, 1987; Smiseth & Lorentsen, 1995*a*). Prior studies conducted on grey seal colonies around Britain have generally supported this bias in investment toward male pups (Smiseth & Lorentsen, 1995*a*). For example, whilst studying the colony at North Rona, Kovacs (1987) observed more time spent nursing, closer proximity and increased effort to maintain this, and higher levels of aggression shown by mothers of male pups than those of female pups. Anderson & Fedak (1987) identified a far higher rate of energy transfer from a mother to her male pup, also on North Rona. In their study, male pup growth rates were, on average, 0.36kg day^{-1} higher than that of females and maternal weight loss rates 0.38 kg day-1 higher in mothers with male pups. Hall *et al*. (2001) observed a bias toward increased maternal investment in male pups on the Isle of May. They identified a greater importance of weaning mass on male pups than female. They postulated that this increased maternal investment should only be supplied by high quality females. Females of low body mass and condition should be expected to invest similarly into male pups as into females pups (Hall *et al*., 2001). Other studies have not found any evidence of bias toward greater investment in male pups. Studies by Smiseth & Lorentsen (1995*a*) in Froan, Norway and Coulson & Hickling (1964) at the Farne islands, Northumberland suggest that gender does not influence

postpartum weaning mass. The former suggests that male pups are simply born heavier than female pups, but growth rates after birth are overall similar (Smiseth & Lorentsen, 1995*a*). The latter shows a positive correlation between growth rate and survival to independence, which is equally important for both sexes. This study identified individual differences in growth rates, but found no relation to the sex of the pup (Coulson & Hickling, 1964). Males tend to be born earlier in the season, with around 3 days between average male and female birth dates on the Farne islands (Coulson & Hickling, 1964) and an average of 5 days on North Rona (Anderson & Fedak, 1987). There is a tendency for larger females to pup earlier in the breeding season, suggesting a possible link between large females and the production of male pups. Indeed, it has been suggested that larger-than-average females, with greater potential for resource transfer, should be more likely to produce male pups (Anderson & Fedak, 1987).

More recently, Pomeroy *et al*. *(*1999) looked at a variety of maternal factors and their relation to pup gender on North Rona. Their research uncovered no effect of pup gender on maternal mass at weaning, overall mass loss and the rate of this loss, duration of lactation, pup birth weight or pup growth rate. Likewise, the age of the mother and date of birth had no link to pup gender. The relative birth mass and weaning mass of pups (as a percentage of maternal mass) was equal in male and female pups (Pomeroy *et al*., 1999), suggesting that maternal physiology may have more of an impact on maternal investment than pup gender.

2.1.3.4 Maternal mass, age and experience

Pomeroy *et al*. (1999) identified a link between maternal postpartum mass (MPPM) and maternal expenditure during pup rearing. All mothers in their study expended approximately 46.5% of their MPPM whilst ashore with a pup. Mothers which over-invested during a breeding season often had reduced fitness in the following year. There was a tendency for pups born earlier in the season to grow more rapidly (Pomeroy *et al*., 1999); this correlates with the tendency for larger females to pup earlier in the season.

An alternative measurement for maternal investment is pup birth mass. Birth mass is often used to estimate pre-natal investment from the mother (Bowen *et al*., 1993). Body mass, age, condition and social rank of mothers have been linked to the birth mass of the offspring across a number of mammals. Studies have been carried out on Sable Island, Nova Scotia, to identify a link between maternal traits and pup body mass and weaning success. These have focussed both on common seals (*Phoca vitulina,* L.; also known as the harbour seal) as well as *H. grypus* (Bowen *et al*., 1993; Bowen *et al*., 2001; Bowen *et al*., 2006). In their 1993 study, Bowen *et al*. (1993) suggest that maternal investment is linked to experience.

Young females with little experience invest less during the pre-natal period, giving birth to smaller pups. Although this reduces the chances of survival for the pup, the mother is able to gain experience of rearing a pup and remaining on a breeding colony without expending excessive energy and limiting future breeding success (Bowen *et al*., 1993). This supports the restraint hypothesis which states that younger individuals should invest less into reproduction in order to conserve reproductive energy for the future (Bowen *et al*., 1993; Bowen *et al*., 2006). A similar outcome was observed during a study by Bowen *et al* (2006) of grey seals on Sable Island. In female grey seals, body mass was found to increase with age. Females tend to give birth to their first pup between the age of 4 and 7 years but, despite this change in female body mass during these years, there is no corresponding increase in birth mass of the first born pup (Bowen *et al*., 2006). This suggests some effect of maternal experience on pre-natal investment.

This difference in investment may extend to behaviour. Young and/or small mothers are expected to show lower maternal expenditure overall (not as a percentage of body mass) than older/larger individuals. In this case, the former may be expected to spend less time interacting with their pup as the physical aspect of this would increase maternal expenditure.

2.2 PROJECT AIMS AND RATIONALE

2.2.1 Project aims

This project aimed to address a gap in current research, identifying whether consistent individual differences (CIDs) are present in maternal behaviours within a wild colony of grey seals using observational and non-intrusive techniques. Whilst CIDs in pup-checking and alert behaviours have previously been identified (Twiss *et al*., 2011; Twiss *et al*., 2012), this study monitored the social aspect of mother-pup behaviour to assess whether play behaviours, seen between juvenile grey seals, occurs between mother and pup, and again determine whether CIDs were present in this behaviour.

Data collected was used to assess changes in aspects of mother-pup interactions during their stay on the colony. The presence of CIDs were tested across broad categories of maternal behaviours from one time period to another (differential consistency).

In addition to this, impact of pup gender and human disturbance on maternal behaviour was considered, measured using a form of mean level consistency. The occurrence of a storm surge and associated extreme high tide presented an opportunity to assess the impact of sudden disturbance on cow behaviour across the colony, and whether exposure to regular disturbance alters this response.

2.2.2 Study rationale

This study builds upon previous successes in the identification of CIDs in grey seals (Twiss & Franklin, 2010; Twiss *et al*., 2011; Twiss *et al*., 2012). CIDs have already been observed in bull behaviour (Twiss & Franklin, 2010), as well as pup-checking behaviour in cows, both disturbed and relaxed (Twiss *et al*., 2011; Twiss *et al*., 2012). These initial findings invite further investigation into grey seal behaviour.

Despite a surge of papers identifying mothering styles in the last decade, the tendency is still toward using unnatural test environments, rather than observations made in nature. The maternal behaviours identified in rodents were based on a series of manipulation tests, whereby the mother was forcibly separated from her pups while the pups were removed from the nest and spread around the cage in order to test the latency to perform a number of maternal behaviours such as nest building and protective crouching (Champagne *et al*., 2007). Other studies have used observations on unmanipulated maternal behaviours, but still using captive bred individuals (for example, Guinea pigs used in Albers *et al*., 1999). Those populations which were observed for maternal styles in the wild were often manipulated in some way, meaning the observation techniques were not entirely non-intrusive. For example, during observations,

all parental individuals in a study of *F. hypoleuca* and all mothers in a study population of free-ranging *M. mulatta* endured regular blood tests throughout the experimental period (Potti *et al.,* 1999 and Maestripieri *et al*., 2009 respectively). The intrusive nature of these studies may have affected the observed behaviour of the parents.

Grey seal maternal styles have received attention in the form of both manipulated and natural behavioural observation. CIDs in pup-checking observed by Twiss *et al*. (2011) were in response to an unnatural stimulus, however more recent pup-check measures were taken using observations of undisturbed seals (Twiss *et al*., 2012). This was still successful in identifying behavioural consistency, suggesting that these consistencies are present during relaxed situations also.

Mother-pup interaction studies on grey seals to date have largely focussed on vigilance behaviours (pup-checking and alert behaviours) and supply of nourishment. Very few have focussed on the social aspect of mother-pup relationships, for example play behaviours. Play behaviours are essential to many species, including humans, allowing infants to develop social responses to signals and practise interactions for adulthood (Bekoff, 1972). Among species with large litter sizes or sociability, infant-infant interaction is often the most important aspect of play (Bekoff, 1972). There is evidence that post-weaning grey seal juveniles play amongst each other and have been noted to have a 'species characteristic invitation to play' (Wilson, 1974). This is characterised by one individual placing their head over the neck or body of the other and resting there for a period. The lower individual may lift their head, bending their neck over that of the upper individual. This is followed by repeated alternations of these positions, occasionally interspersed with a 'leaping' action in which one individual lifts the main trunk of their body on top of the other. This play invitation continues throughout the interaction, and if neither perform these behaviours, interaction will cease (Wilson, 1974). However, grey seal mothers are intolerant of other individuals interacting with their pups prior to weaning. This includes both adults and other pups (Wilson, 1974). So, while still dependent on their mothers, pups are unlikely to make contact with other pups on the colony in order to develop this 'invitation' behaviour. It is possible that this characteristic play behaviour is developed between mother and pup prior to weaning. This intolerace makes the study of personalities in grey seals particularly interesting. As mentioned previously, the timing at which an event occurs in an individual's life may alter its impact on behavioural development (Sih *et al*., 2004). The lack of sociability in the early stages of a grey seal pup's life means that early experiences are likely to be very different from pup to pup. If personality is

built from these early life experiences, interactions with the mother may have a large impact on pup behaviour and the appearance of different personalities. However, prior to assessing the potential impacts of mothering styles on seal pup development, it is essential that the presence of mothering styles be confirmed through identification of CIDs in grey seal maternal behaviours. This is the purpose of the current study

2.2.3 Methodology rationale

Donna Nook is located on the Lincolnshire coastline (more information in section 3.1.1). The combination of terrain and accessibility for the seals is dissimilar at this site to others identified in the UK. Redman *et al*. (2001) describe two common breeding grounds in the UK. The first, inland sites located on small, offshore islands, often with rocky terrain and poor access to the sea. The second, beach sites with unrestricted access to the sea. Donna Nook has the terrain of a beach site, but similar restrictions as found on an inland site. The sea is often around one mile from the inner breeding colony and, as such, locomotion to reach the water source is costly, similar to the island sites. Prior studies report that more time is spent performing mother-pup interactions on terrain with easier access to the sea than on inland sites with few access points (Twiss *et al*., 2000). These differences necessitate the study of grey seal behaviour on this site to identify into which category, if either, Donna Nook falls. The pupping season is comparatively late. The average pupping date for most sites around the UK falls between September and October. The breeding season at Donna Nook is similar to that of the Farne Islands, beginning toward the end of October and continuing into late December and occasionally into early January (Coulson & Hickling, 1964).

The colony at Donna Nook began in the early 1970s and is rapidly increasing from year to year (Abt & Engler, 2009). Pup production alone is increasing by around 15% per year (Thompson & Duck, 2010). It is postulated that the growth of this colony may be linked to recruitment from declining colonies such as the Farne Islands and Isle of May. This speculation is supported in part by the identification of individuals at Donna Nook with flipper tags, originating from the Isle of May (Pomeroy *et al*., 2010; Thompson & Duck, 2010). A section of the breeding colony along the Donna Nook coastline is exposed to an estimated 70,000 human visitors over the course of a single breeding season (Thompson & Duck, 2010). This provides an opportunity for assessment of the impacts of human disturbance on maternal behaviour. Public access to the colony is permitted along a footpath; humans and seals are separated by way of a coutryside type fence (erected in 2007). However, this access is limited to only one section of the colony. The other section of the colony falls along a Ministry of Defence (MOD) owned beach, blocked from public access. Mother-

pup pairs located along the public access stretch of the beach were exposed to noise and visual disturbance, both of which fluctuate in intensity but are a constant presence throughout daylight hours. Mother-pup pairs on the restricted beach were not exposed to this disturbance, providing an excellent opportunity to study the impact of human disturbance on maternal behaviours within the same colony.

Data collection methods were selected based upon methodologies described in previous studies of maternal behaviour, and a prior knowledge of maternal behaviours among grey seals. A number of studies concerning both the identification of CIDs in basic behaviour traits, as well as those in maternal behaviour, involve the use of video recordings, known as focal videos. These focal videos are analysed at a later date, in conjunction with pre-determined behavioural ethograms in order to extract the required data. Focal videos have been used to identify mothering styles in a range of taxa, from birds (Nakagawa *et al*., 2007), and primates (Lee, 1984) to marine mammals, including dolphins (Hill *et al*., 2007). Indeed, Twiss *et al*. (2011 and 2012) used focal videos in order to identify CIDs in grey seals. Although scan samples have previously been used to create time budgets (Twiss *et al*., 2000; Twiss & Franklin, 2010), it was felt that these would not be suitable for this study. In order to perform a scan sample, the behaviour of known individuals is recorded instantaneously at equal intervals, for example, every five minutes. Some maternal behaviours, however, last only a few seconds and, as such, a scan sample may not pick out these behaviours. In addition to the focal videos, pup-check counts and proximity maps were used. Pup-check behaviour has previously been shown to be repeatable (Twiss *et al*., 2011; Twiss *et al*., 2012). However, a single pup-check can be fast and difficult to identify or confused with an alert behaviour if the exact location of the pup is unknown. For this reason, an in-field method of evaluating pup-check frequency was used. Mother-pup proximity was to be estimated by eye, in adult seal body lengths (bl; c. 2m). It was felt that on-site collection of this data would be more reliable than that obtained at a later date from the focal videos. For this reason, proximity maps were used whilst in the field, recording both mother-pup proximity and number of individuals within five body lengths of the focal mother.

Other categories used to identify CIDs in maternal behaviours were selected based on their use in previous studies. Affiliative and agonistic behaviour toward offspring and maternal locomotion were found to be repeatable in a number of species, including guinea-pigs (Albers *et al*., 1999) in which they have been linked to lasting effects on offspring behaviour (Albers *et al*., 2000*b*). This is true also of a number of primate species (Bardi & Huffman, 2002; Weaver & de Waal, 2002; Bardi & Huffman, 2006;

Maestripieri *et al*., 2009). Female grey seal locomotion, with respect to their pup, has previously been divided into short distance locomotion (less than 5 bl) and long distance locomotion (greater than 5 bl). However, the majority of locomotion was found to remain within the short category (Redman *et al*., 2001). This provides rationale for the further division of the short locomotion category within this study, in particular for on-site proximity mapping. Play behaviour was of particular interest, having been identified between juveniles of the species. This accounted for a proportion of the affiliative behaviours category.

In addition to the comparison of individual behaviours observed in the field, this study uses a measure of mother-pup relationship quality adapted from Weaver and de Waal (2002). The Mother-Offspring Relationship Quality (MORQ) index, in its original form, was used to describe the quality of relationship between mothers and offspring in a family group of brown capuchin monkeys. This was based on a ratio of affiliative to agonistic behaviour seen between the mother and her young, calculated relative to that of all other mother-young pairs (with young of the same age) within the colony. This provides a value for each mother-young pair which can be compared across the colony and used to identify pairs with a more affiliative relationship and those with a more agonistic relationship relative to the colony as a whole. This was adapted to fit grey seal maternal behaviours and provided numerical values by which mothers could be compared.

It is essential that each study of personalities define their use of context and time period over which CIDs are being analysed. Data collection was limited to a single context - pup rearing. Mother-pup interaction ceases abruptly after weaning and does not continue into other contexts. Within the context of pup-rearing, two time periods were used by the current study: early lactation and late lactation (as used by Iverson *et al*., 1993). The definition of personality used for this study is therefore CIDs across time alone. As a number of breeding periods could not be included in the analysis, the results do not fit the definition of mothering styles (CIDs over a number of lactation periods). However, the analysis focusses on the identification of CIDs in mother-pup interaction behaviours, rather than more general behaviours such as boldness, shyness, exploration or aggression. In this way, the study combines methods from personality and mothering style research. Similar methods have been employed by Lee (1984) who used two focal videos from the first 12 weeks of infant life to identify CIDs in maternal behaviours over a single lactation period, and Hill *et al*. (2007) who extract mother-offspring behaviour from dolphins for only one of four years during which this bond is present.

The results of this study will provide preliminary evidence to either support or reject the existance of mothering styles in grey seals, upon which further research can be built.

2.2.4 Research questions

1. Does the proportion of time spent on certain maternal behaviours change between early lactation and late lactation?

    a. Are there consistent individual differences (CIDs) in behaviour between mothers, when compared between early lactation and late lactation.

    b. Are some mothers consistently more affiliative than others? Does this affiliative behaviour (or rejective behaviour) correspond with pupping date? If so, can differences in maternal behaviour be linked to estimated maternal experience (as more experienced mothers are expected to pup earlier in the season)?

2. Does the gender of the pup influence maternal time budgets?

3. Is there an observable impact of regular human disturbance on maternal time budgets?

4. Is there an observable impact of sudden environmental disturbance on maternal time budgets?

    a. If so, are these changes in behaviour mitigated or exacerbated (if changed at all) by prior exposure to the regular disturbance?

3. METHODS

3.1 IN-FIELD DATA COLLECTION

3.1.1 Study site background

The field season took place between November 8th to December 10th 2011 on Donna Nook. Donna Nook is situated along the Lincolnshire Coastline, UK (OS Grid Reference: TF422998). Covering over 6.25 miles of coast, Donna Nook is a Lincolnshire Wildlife Trust nature reserve. A section is held by the Ministry of Defence (MOD) for target and bombing practise. **Figure 3.1.1** shows the study area in full, however only a small subsection was used for data collection for this project. Access to the coastal path was gained in two areas: the first via the public access entrance from the Stonebridge car park, the second via an MOD entrance. These are shown by the red dotted line within the boxes in **Figure 3.1.1**. From hereon these sites are denoted Public site and RAF site respectively. Access by the general public was restricted by a fence between the public and RAF sites, however the breeding colony was continuous between the sites.

Topography on both sites was fairly similar. Both were mostly flat and continuous with no rocks. However, the public section consisted of a combination of silty wallows, grass banks and gullies. This combination of terrain on the public site provided a number of sheltered areas and ditches in which individuals were able to rest, and which periodically filled with water from rain and/ or incoming tidal flow deep enough to allow swimming. Terrain on the RAF site was more consistent, a flat sand bed, bordered by both natural and man made sand dunes.

3.1.1.1 Observation points

The stretches of coastline from which observations were made are shown in Figure **3.1.1** by the satellite images. Observations were made on foot from various points along the public footpath on the public site, and the dunes on the RAF site (both marked by dashed lines on satellite images). On the RAF site, observations were made while seated (where possible), and when available, the marram grass to the rear of the dunes was used as cover to minimise disturbance. Seated observations were not possible on the public site as a double fence separated the public from the breeding grounds. The combination of this fence and the public along the footpath would cause the seated view to be obstructed. However, disturbance caused by movement of the observer alone was minimal in this area as visitor numbers were regularly in the hundreds, causing continuous noise and visual disturbance throughout daylight hours.

Observer positioning was particularly important as a clear view was needed of all

focal animals for that day. This further reduced any disturbance caused by the movement of the observer. Wind conditions were taken into account, ensuring that the observer was positioned downwind of focal animals. This avoided novel scents affecting the natural behaviours of the seals. Observations were never taken from below the dunes on the RAF side and the fence was never crossed on the Public side. This kept observer contact with the colony to a minimum and reduced intrusive disturbance to the colony, as grey seals are particularly sensitive to new sounds, smells and movement (Fogden, 1971).

Data collection took place between the 10th of November and 10th of December 2011. November the 8th and 9th were used for making general observations, while improving methods of data collection and individual seal recognition. During these first two days, the presence of the 'characteristic invitation to play' was observed between mothers and their pup, and was therefore included in the study as an element of 'rolling' (see **Table 3.2.1**).

3.1.2 Focal animal identification

3.1.2.1 Identification of females

Females with young pups (either stage 1 or 2, see section 3.1.2.2 for definition of pup stages) were identified initially. Those with clear, recognisable markings on at least one flank were favoured as this made future identification more likely. Females were selected in the same way on both the public and RAF site. An identification number (referred to as Seal ID) was given to each female, prefixed with the letter corresponding to the site at which the seal was found (**P** = public; **R** = RAF. LINDA is the only exception to this, identified as such due to an 'LI' marking on her side. This female was located on the Public site, her ID remained an exception to separate her as the only female with a known pupping date and whose first focal video was taken on the hour of birth). Although the breeding colony was continuous between the RAF and public sites, no identified females were found moving between sites during the course of the study. Sketches were made of striking markings, showing position and orientation on the body. Photographs were taken of each female, using a Canon EOS 350D digital camera capturing both sides of the body as well as dorsal and ventral surfaces where possible. Photograph number was recorded in a notebook along with the Seal ID given to the female. Photographs were later collated into a photo-catalogue on a second generation iPod Touch (Model: MB531BT; Version 4.2.1 (8C148)) and kept in individual folders labelled with the Seal ID.

Females were identified again later in the breeding season using these resources. It was imperative that identifications be accurate. Incorrect identification of females would eliminate the possibility of correctly identifying any consistent individual

differences (CIDs), if these were present. After sightings were made, clear markings were matched to existing sketches and the female was assigned a preliminary Seal ID based on this match. The smaller and more difficult to decipher markings were then matched to photos in the iPod Touch photo-catalogue. Once positively identified, sketches were expanded and improved, and new photos taken for the photo catalogue. Data collection only began after this conclusive identification.

3.1.2.2 Classification of pup stage

Pup stages were classified according to descriptions found in Bowen *et al*., 2003. The main characteristics looked for are summarised here:

Stage one: (**Figure 3.1.2** *a* & *b*) Visibility of ribs and hips; fresh umbilicus; lack of general co-ordination; yellow tinge to lanugo. Approximate age 1 - 3 days.
Stage two: (**Figure 3.1.2** *c* & *d*) Layer of blubber reduces visibility of ribs and hips; umbilicus darkened and dry or lost; co-ordination improved but still not fully developed; white lanugo. Approximate age 4 - 8 days.
Stage three: (**Figure 3.1.2** *e* & *f*) Barrel-shaped body; much improved co-ordination and movement; may be some loss of lanugo on extremities (muzzle and flippers) but full lanugo still on body. Approximate age 9 - 14 days.
Stage four: (**Figure 3.1.2** *g* & *h*) Moulting of lanugo to juvenile pelage in places about the body other than the muzzle and flippers, but not fully complete. Approximate age 15 - 17 days.
Stage five: (**Figure 3.1.2** *i* & *j*) Full moult of lanugo to juvenile pelage complete. Approximate age 18 + days.

Where possible, in addition to pup stage, the sex of the pup was determined. Male and female pups were distinguished by the presence or absence (respectively) of a penile opening. This was identifiable as a small opening located on the ventral surface, midway between the navel and hind flippers, the presence of which becomes obvious at around stage 3 making differentiation between male and female possible. As data collection methods were observational and not intrusive this was not always possible. Often pups were orientated with the ventral surface against the ground or too far away to make an assessment.

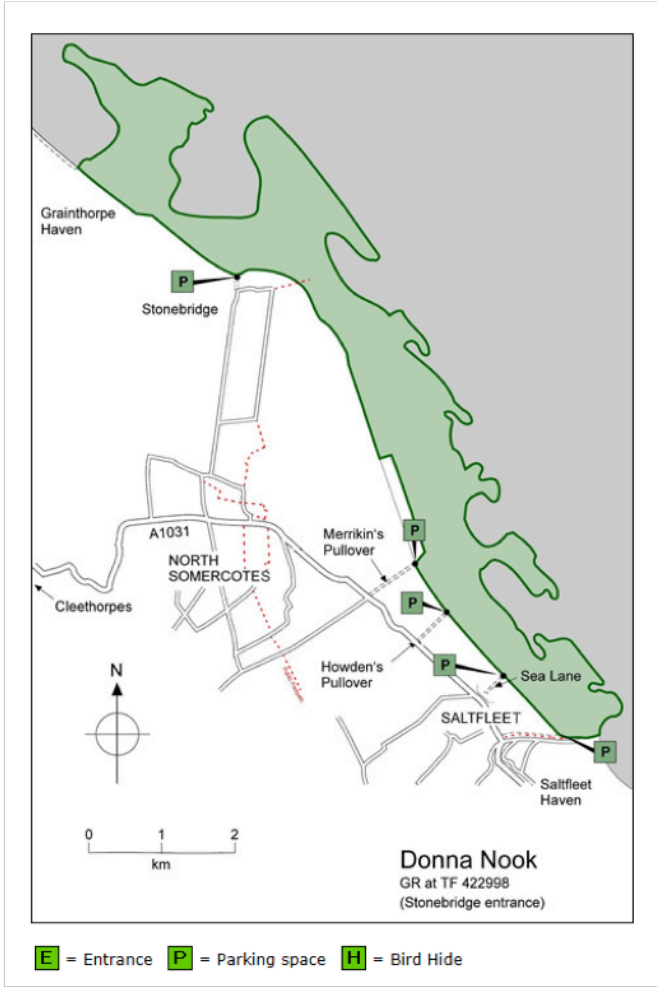


Figure 3.1.1 Map showing the Nature Reserve area along the Donna Nook coastline. Available from http://lincstrust.org.uk/reserves/nr/print-reserve-maps.php?mapref=15. Satellite images, taken from Google Earth, show the stretch of each part of the coastline used for observations. The public site (left) and RAF site (right).

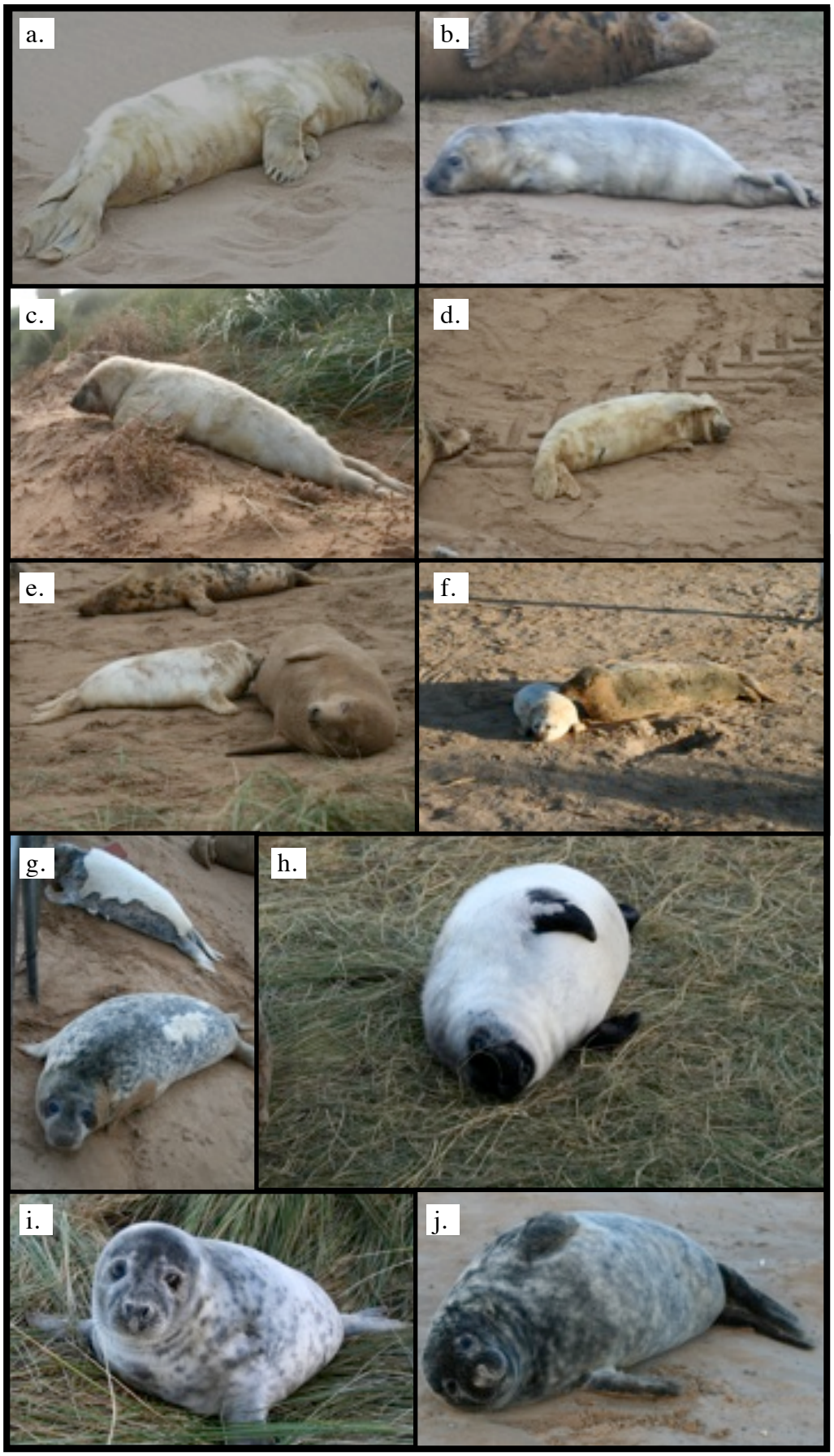


Figure 3.1.2 Different pup stages at Donna Nook: a & b. Stage 1 (b. shows natal pelage colour when juvenile undercoat is black); c & d. Stage 2 (note difference in colour of umbilicus between photos a. and d.); e & f. Stage 3; g & h. Stage 4 (h. shows pup with black juvenile undercoat); i & j. Stage 5.

3.1.3 Behavioural data collection protocols

Data collection consisted of a combination of focal videos, in-field pup-check counts and proximity mapping. Pup-checking behaviour is described in **Table 3.2.1**. As this project aimed to identify CIDs across time (and to an extent mothering styles), a reasonable length of time had to pass between initial and subsequent data collections. Females were allocated a 'lactation stage' according to the age of their pup; these were based on classification of lactation stages given by Iverson *et al*. (1993). Females with stage 1 and 2 pups were categorised as 'early lactation', these pups had little co-ordination and so interactive behaviours were expected to be minimal. Females in 'late lactation' had pups at either stage 3 or stage 4. These older pups had more muscle co-ordination and play behaviours were expected to increase. Initial data collection took place on females categorised as early lactation; subsequent data collection did not take place until the female was classified as late lactation. A preliminary timetable was organised, in which the second set of data collection took place eight to sixteen days after the first. However, as the speed at which the pups developed varied from individual to individual, decisions were made daily regarding whether the pup had grown sufficiently for subsequent data to be collected. Where possible, an additional focal was taken. Only two were required for statistical analysis, however, this third focal prevented a bias toward a high number of stage 3 pups in the results. Third data sets were collected from mothers with stage 4 pups, already having data collected from early lactation and at pup-stage 3. When analysed, a Microsoft Excel randomiser was used to select one video from stage 3 or 4 for these females. This ensured that, when assessing behavioural categories across the colony, there was a spread of data from the two stages of late lactation.

Focal videos were taken using a Canon DC40 mini DVD camcorder, recording onto mini digital versatile disks. Each video focal was approximately 30 minutes; the length of a disk. 30 minutes was considered a suitable length for focal videos for this preliminary investigation into CIDs, based on methodologies in similar studies (for example, see Twiss *et al*., 2000; Hill *et al*., 2007; Twiss *et al*., 2012). There are limitations to the data as a result of these short sampling times. These are taken into account during the discussion.

Two kinds of focal videos were collected from each female. Random focal videos were collected in the morning (between 7 am and 12 midday). Focal females for that day were identified (see section 3.1.2.1) and a random focal began of the first female to move toward her pup (scheduling method from Altmann, 1974) ensuring sampling time was random. Nursing focal videos were taken as a mother and pup began to show pre-nursing behaviours such as flippering and

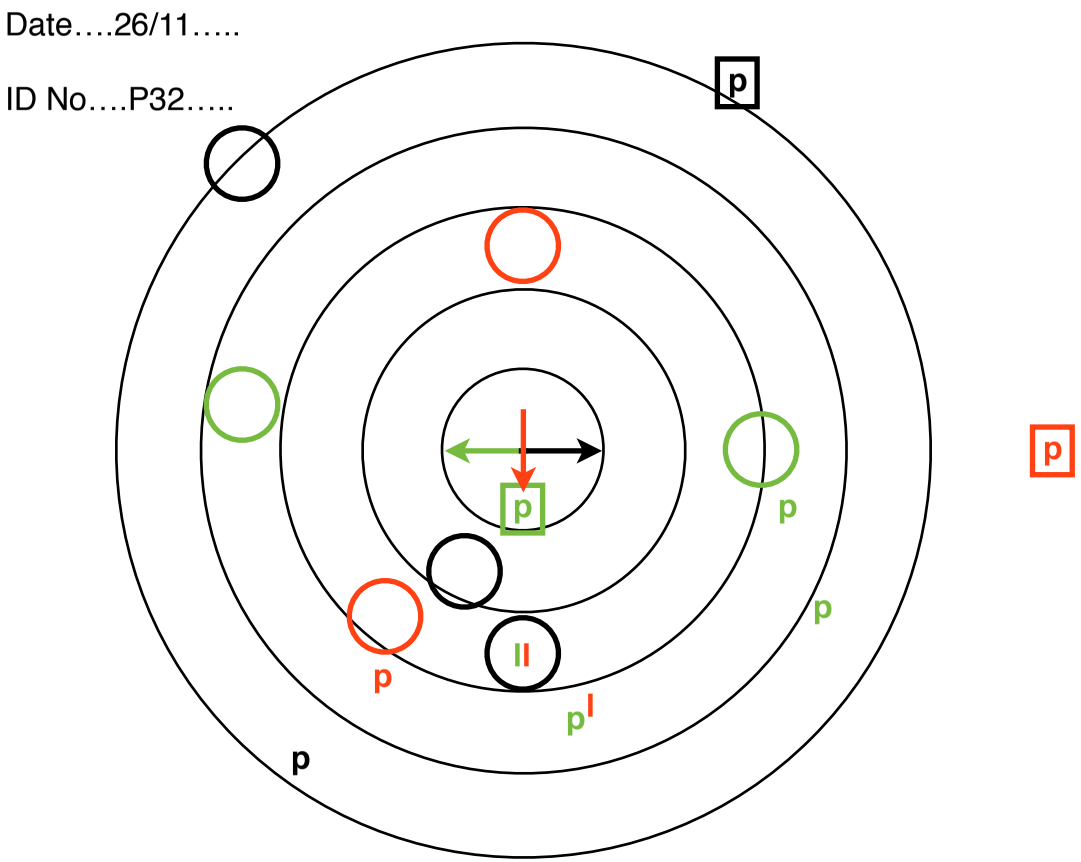
presenting. Any random focal video during which a female began nursing her pup was considered a nursing focal.

During the afternoon (12 midday to 5 pm) proximity maps were drawn and pup-check counts collected from each female from whom focal videos had been taken in the morning session. Proximity maps and pup-check counts were taken hourly, beginning by mapping the number of individuals within a 5 seal-body length (bl) radius of the focal female and the distance in body lengths between the female and her pup. This was followed by a 15 minute in-field count of the total number of pup-checks made by the focal female.

The number of individuals, and their position within 5bl of the focal female, were mapped three times per afternoon. Each mapping was separated by an hour. **Figure 3.1.3** shows an example of one map taken of female P32 on the Public site. The focal female was always located at the centre of each map, and her pup's location was denoted by a P enclosed in a square. The different colours represent different counts throughout the same afternoon; marks in black are the first counts, green represents counts taken one hour from the start, and red represents those taken after two hours. A limit of 5bl was decided upon, as previous studies have suggested that mothers rarely moved more than 5bl from their pups (Redman *et al*., 2001). Distances in bl were estimated by eye, using the focal female's body as a reference. The observation point was located above the colony, giving a good view of the surrounding area and ensuring no individuals were overlooked or hidden.

Additional overall population counts (including all individuals on the colony, classified into females, males and pups) were obtained from the wardens at the end of the season.

In-field pup-check counts were taken for 15 minutes after a proximity map had been drawn. The female was observed continuously and the number of pup-checks tallied. As with proximity maps, these were taken 3 times during the afternoon with an hour gap between each.


Date….26/11…..
ID No….P32…..
p
p
p

3.2 EXTRACTION OF BEHAVIOURAL DATA

In order to extract data from the focal videos, an ethogram of broad behavioural categories (including finer categories for mother-pup interactions) was created. This used a combination of in-field observations and published ethograms (Fogden, 1971; Wilson, 1974; Kovacs, 1987; Twiss *et al*., 2011). The ethogram used throughout this study can be seen in **Table 3.2.1**. These behaviours were used to create a visual macros programme in Microsoft Excel (Microsoft 2010; **Figure 3.2.1**). Each behaviour listed in the table was given a separate 'button' which, when clicked, entered a start time for that behaviour into an excel table. Once a subsequent behaviour button was selected, an end time was entered for the previous behaviour. This was used whilst watching the focal videos. Using the start and end time of each behaviour, the overall duration of the focal spent performing that behaviour could be calculated.

A single table for each female was produced using Numbers '09 (version 2.0.5 (368)). Total duration of each behaviour per focal was translated into the percentage of each focal (POF) spent performing each behaviour.

In-field pup-check counts and data from proximity maps (number of individuals in 5bl proximity; distance between mother and pup) were collated into one spreadsheet using Numbers '09. Pup-check counts were used throughout the analysis along with data from the focal videos. In-field pup-check counts were used in their raw form (as three counts of the number of pup-checks made in 15 minutes). The three separate counts were combined and divided by 45 to provide an in-field pup-check rate per minute.

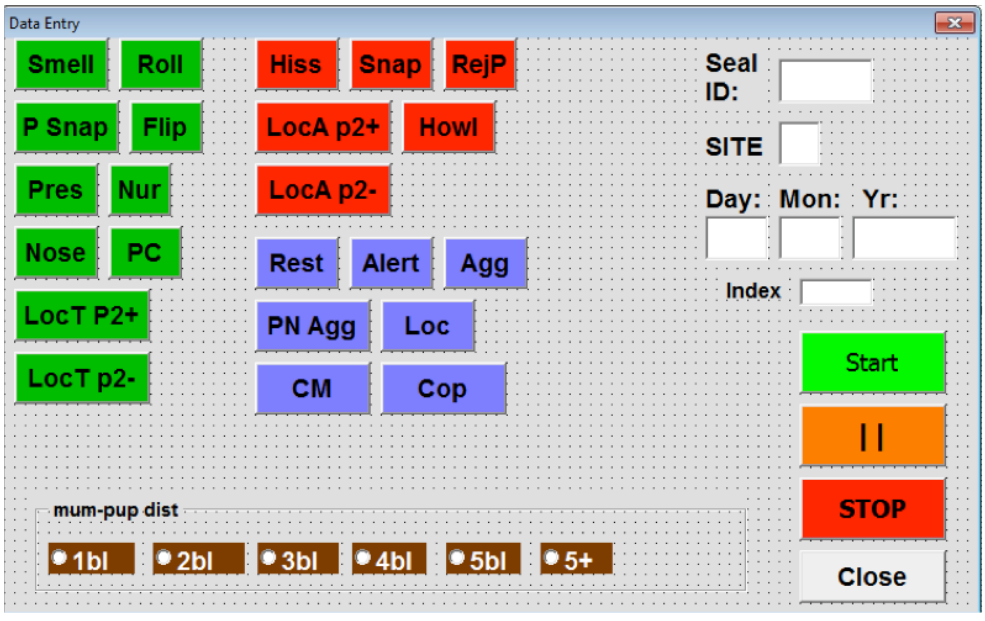


Figure 3.2.1 Screen shot of the data entry window used in Microsoft Excel. This was used in conjunction with the video focals to convert visual data into behavioural time budgets.

Table 3.2.1 Ethogram describing the behaviour of focal females. Classification identifies whether the behaviour has a social aspect with respect to the pup (either Affiliative or Rejective) or is not a mother-pup socialisation (Other). Proximity is the distance in adult body lengths between mother and pup at any given time. The column labelled MORQ categorises behaviours according to whether they were included as affiliative behaviours (**A**) or rejective behaviours (**R**) when calculating MORQ index. Blank means behaviour was not included.

Behaviour	Description	MORQ
Roll	Mother rolls her body in contact with the pup. This can either be a partial roll (laying on back, body pressed against pup) or a full roll (one or both fore-flippers over pup, body on top of the pup).	**A**
Play Snap	During an interactive bout, the mother gently snaps at the pup. Usually slower than a rejective snap, and full closure of the jaw by the mother is rare.	**A**
Smell	The mother presses her nose against any part of the pup's body and sniffs.	**A**
Nose	Mother and pup press their noses together. Often followed by both heads being lifted from the ground and moved from side to side, noses still in contact (also called nuzzling by Fogden, 1971).	**A**
Pup Check	The mother lifts her head to make a direct, aimed look at the pup.	**A**
Flipper	The mother repeatedly strikes her flipper against the pup. This is used for many purposes: to encourage the pup to feed; in play situations whereby the pup snaps at the flipper; more viciously used when the pup is in danger during aggression with other adults (i.e.. To push the pup out of the way).	**A**
Present	The mother lies on her flank, exposing her nipples to the pup. For the purpose of this project, presenting was defined as when the pup actively nosed at the nipple. This was for two reasons: it was not always clear when the female was directly presenting to the pup, or whether she was simply resting on her side and by defining presenting in this way, the time spent nosing at the nipple by the pup could be compared between individuals.	**A**
Nurse	The pup makes direct oral contact with the nipple, latching on and suckling.	**A**
Locomotion toward pup (>2bl)	The mother uses her fore-flippers, or performs a full body roll, moving geographical location toward the pup whilst being over 2 body lengths away from the pup. This includes any locomotion involved in the initial stages of aggression.	
Locomotion toward pup (<2bl)	The mother uses her fore-flippers, or performs a full body roll, moving geographical location toward the pup whilst being 2 body lengths or less away from the pup. This includes any locomotion involved in the initial stages of aggression.	

Table cont. overleaf

Table 3.2.1 cont.

Howl	The mother emits a loud howl, directed at the pup. Often when alarmed.	**R**
Hiss	The mother opens her mouth and emits a gentle hissing sound directed at the pup. Usually combined with a snap.	**R**
Snap	The mother bites sharply at the body, head or flippers of her own pup. This can be distinguished from a play snap by the speed (faster than a play snap) and the intensity of the bite (the jaw may be fully closed at the end of the snap, and it may be accompanied by a howl or hiss).	**R**
Reject pup contact	The pup attempts to make contact with any part of the mother's body. The mother sharply retracts or relocates this body part, or fully moves away from the pup without first performing a pup check.	**R**
Locomotion away from pup (>2bl)	The mother uses her fore-flippers, or performs a full body roll, moving geographical location away from the pup whilst being over 2 body lengths away from the pup. This includes any locomotion involved in the initial stages of aggression.	
Locomotion away from pup (<2bl)	The mother uses her fore-flippers, or performs a full body roll, moving geographical location away from the pup whilst being 2 body lengths or less away from the pup. This includes any locomotion involved in the initial stages of aggression.	
Rest	The mother is in a relaxed state with her head close to or touching the ground.	**R**
Comfort Move	The mother readjusts her body, scratches or shakes any part of her body or smells the ground around herself.	**R**
Alert	The mother lifts her head to look at the environment. This can be either a directed look at perceived danger or a scan of her surroundings. This does not include a direct look at her pup (see Pup Check).	
Locomotion	Same definition as other locomotion categories. Used when locomotion had no clear directionality with relation to the pup.	
Aggression	Any form of aggressive behaviour with *other* pups (not her own) or adults on the colony. Includes open mouth threats, howling, hissing, flippering, snapping or biting.	**R**
Post Nursing Aggression	As above, however this occurs immediately after a nursing bout ends and is always initiated by the mother. The mother will break contact with the pup (she may briefly smell her pup first) and proceed to show *unprovoked* aggression to other individuals.	**R**
Copulation	This includes both unsuccessful and successful copulation attempts. A male approaches, and grips the mother. He may place his body over hers to begin with. A successful copulation occurs when intromission is achieved.	**R**
Drink	The mother clearly drinks water from a puddle, pool or wallow.	**R**
Distance	The distance between mother and pup in seal body lengths (1 body length = c.2m).	

3.3 STATISTICAL ANALYSIS

Data collection during late lactation required confident identification of females with data from early lactation. As this was not always possible, there were a number of females which were observed once but could not be re-identified. For the purpose of this study, only females with two or more focal videos were included in the statistical analysis. Data were split into early and late lactation stages. As only 2 data sets were required for analysis, a randomiser was used to select just one early lactation and one late lactation focal video, pup-check scan and proximity map from any female with more than one of each.

Maternal behaviours were placed into broader categories based on previous literature. These were:

- *Play*: Play behaviours include the characteristic 'roll' based upon the species specific play invitation described in section 2.2.2, nosing and play snap.
- *PINT*: This stands for Mother-pup interaction and includes both positive and negative physical interactions - nose; play snap; present; roll; flipper; smell; snap; howl; hiss. Nursing is not included in this category.
- *PINT + nursing*: This is the same as PINT, but includes nursing. This data was only obtained from nursing focal videos.
- *Nursing*. This data was only obtained from nursing focal videos.
- *Alert*.
- *Pup-check*.

All of these categories were measured as a percentage of the total focal time (POF), calculated as a summation of POF of all behaviours in that category.

In addition to comparing individual behaviours, or groups of these behaviours, a Mother-Offspring Relationship Quality (MORQ) index was calculated for each mother-pup pair with nursing and/or random focal videos from both early and late lactation. Although originally designed as a measure of affiliative versus agonistic behaviour, initial observations of mother-pup interactions in grey seals were found to be rarely agonistic and more frequently rejective. For this reason, in this study the MORQ index refers to the ratio of positive mother-pup interaction to rejective behaviours. **Table 3.2.1** categorises behaviours used in the MORQ index into affiliative (A), rejective (R). Those behaviours left blank in the MORQ column were not used in this calculation. "Rejective" behaviours were selected on the basis of time the mother chose to spend solitary or interacting with others rather than interacting with the pup. This provided a contrast

between mothers who frequently spent time socialising with their pup, and those who spent little time interacting with their pup at all, focussing their time away from their pup. Mothers frequently rested despite their pup being awake and active. They would also spend time interacting with other members of the colony as well as performing solitary behaviours such as leaving the pup alone in order to drink. Locomotion and alert behaviours were not included in the MORQ index as it was not always clear whether this behaviour was centred around pup protection or interaction. One MORQ index value was calculated from each focal video. The POF spent performing affiliative behaviours and the POF spent performing rejective behaviours was calculated for each focal video. Average affiliative and rejective POFs were calculated for all other mother-pup focal pairs of the same lactation stage. The mother's affiliative value was then divided by the average affiliative value for the other mothers, and her rejective value divided by the average rejective value calculated from the other mothers. This gave affiliation and rejection values relative to the colony. Finally, the relative affiliative value was divided by the relative rejective value in order to give a ratio of affiliation to rejection, relative to other mother-pup pairs at the same lactation stage.

The equation used to calculate the MORQ index for each female was:

$$\frac{(\mathbf{FA} / \mathbf{CA})}{(\mathbf{FR} / \mathbf{CR})}$$

Where:

FA = POF spent on affiliation by focal female

CA = average POF spent on affiliation by other females with pups of the same age

FR = POF spent on rejection by focal female

CR = average POF spent on rejection by other females with pups of the same age

Nursing focal MORQ index values were compared to stage 2 pup dates, as well as in-field pup-check rates and mother-pup proximity measures. MORQ values and in-field pup-check rates were separated into early and late lactation. A Spearman's Rank correlation (used because of the small, non-parametric data set) was calculated between early MORQ values and early in-field pup-check rates. This was repeated for the late values.

An average of early and late lactation MORQ index values was then calculated for each female, and examined in relation to the number of days after November 1st 2011 her pup was recorded as a stage 2. These dates acted as an approximation for pupping date relative to others as exact pupping dates were not available for the majority of mothers in this study. However, all mothers with MORQ index values had known dates at which the pup was first at stage 2.

In-field mother-pup proximity measures were calculated from proximity maps. This

provided 3 measures of proximity from early lactation and 3 measures from late lactation. The number of occasions (from these 3 measures) that mother and pup were observed within 1bl of each other was compared to MORQ index values, again separated into early and late lactation. Comparisons were made with Todman & Dugard (2001) design 5 for small groups.

3.3.1 Identifying consistent individual differences (CIDs) in maternal behaviour

Prior to calculating the repeatability of behaviour between early and late lactation, a two-tailed randomisation test was used to identify any effect of lactation stage on maternal behaviour. This MS Excel based program (Design 5a from Todman & Dugard, 2001) compares two samples with small, uneven data sets - a reason for its selection over a more standard Mann-Whitney U test. The program calculates the mean of each sample, then rearranges data points across samples (maintaining the original sample size) 1000 times. Test significance is determined by the number of these rearrangements in which the mean difference is greater than or equal to the mean difference of the original samples. A Mann-Whitney U test was selected to assess the in-field pup-check rates as the sample size was larger for this category. This test was carried out using Minitab 16 Statistical Software (2010).

All repeatability measures were made using the statistical software R, version 2.14.1 (R Development Core Team, 2011). Though a number of repeatability measures have been used to identify CIDs in personality traits, the intraclass correlation coefficient (ICC) was selected for this study. The ICC is ideal for this study as it identifies both inter- and intra-individual variation, producing a ratio of variation within a population to an individual's phenotypic variance. From this ratio, the presence of CIDs is confirmed if phenotypic variation is low and population variation is higher (Hayes & Jenkins, 1997). This follows on from previous studies using the ICC to identify CIDs in grey seals (e.g. Twiss *et al*., 2011). During the current study, the ICC was used to identify CIDs within the broader categories of maternal behaviour listed at the beginning of this section (3.3), as well as between MORQ values, in both nursing and random focal videos. The ICC compared measures from mothers between early lactation and late lactation focal videos.

3.3.2 Identifying possible drivers of maternal behaviour

Various external factors have previously been linked with differences in maternal attendance. Amongst these, two were selected for comparison within this study. Firstly, the effect of pup gender and secondly, the effect of environmental disturbance. The study site proved highly suited to test the latter of these factors. Firstly, behaviours were compared between the two sections of

the beach - the public site and the RAF site. The daily presence of human visitors formed regular noise and visual disturbance at the public site. Members of the colony on the RAF site (which had no public access) were not exposed to this type of disturbance. Further to this regular disturbance, there was an unexpected storm surge on November 28th, which caused an exceptional high tide. This caused a massive disturbance to all individuals on the colony, both on the public and RAF sites. Many females with pups were separated. Many individuals deserted the beach; remaining members of the colony positioned themselves further inland, away from the tide-line. This sudden disturbance provided an interesting opportunity for assessing the behavioural changes of grey seal mothers.

3.3.2.1 Pup gender

Pup gender was determined in the field. Broader categories of maternal behaviour were compared between mothers of both genders, again using Todman & Dugard's (2001) randomisation test for small samples. This compared behavioural measures (POF and rates) between early and late lactation for mothers with male pups and mothers with female pups separately to ensure that lactation stage had no effect on the measures. Early and late measures were then combined for mothers with male pups and mothers with female pups separately and one measurement from each female selected using a randomiser. This ensured no bias towards a single female or a certain lactation period in the data set. This method also increased the sample size. Behavioural categories were then compared between mothers with male pups and mothers with female pups using the randomisation test for small samples (Todman & Dugard, 2001). Nursing and random focal videos were kept separate throughout this process

3.3.2.2 Regular disturbance

Maternal behaviours of mothers on the public site and on the RAF site were compared against each other in much the same way as those with pups of different gender. First, early and late lactation measures were compared within each site to ensure no effect of lactation stage. Then measures were combined, and one selected at random from each female. Measurements were compared using the randomisation test for small samples (Todman & Dugard, 2001). Nursing and random focal videos were kept separate throughout this process.

3.3.2.3 Sudden disturbance

The storm surge and resulting extreme high tide provided a sudden and unexpected disturbance on both the public and RAF sites. POF spent on broad behaviour categories were compared between nursing and random focal videos, first from the period prior to the storm surge (pre-tidal) and then from after the surge (post-tidal) using Todman & Dugard's (2001) randomisation test. As there was no significant difference in POF spent on

any behaviour category between nursing and random focal videos during either pre- or post- tidal periods, this analysis combined these. Pup-check counts and rates taken in the field were analysed. One focal date was selected from each female (two from those with dates which lay either side of the storm surge - one from before and one from after the Nov 28th). In addition, counts of the number of individuals within a 5bl radius of the focal female were compared between pre- and post-tidal periods The two-tailed randomisation test from Todman & Dugard (2001) was used to compare pre- and post-tidal POFs. Again, due to the larger sample size of in-field data, a Mann-Whitney U test was conducted in Minitab 16 to compare pre- and post-tidal in-field pup-check rates and number of individuals in a 5bl radius. Further to this, data was compared between public and RAF females identifying any differences in behavioural levels either prior to the storm surge, or after this disturbance occurred. This aimed to identify any differences in coping strategies in response to a sudden disturbance between mothers exposed to regular disturbance (public) and those not (RAF). Though multiple regression techniques would have proved ideal for the comparison of pre-tidal and post-tidal data between the public and RAF sites (given the inclusion of several independent variables), the data set available for this comparison did not contain sufficient observations to allow the use of this analysis. A minimum of five observations for every independent variable is necessary for a reliable multiple regression analysis (Brace *et al*., 2002). Instead, focal data were compared using Todman & Dugard's (2001) randomisation test, and Mann-Whitney U in Minitab 16 for the in-field pup-check counts, which had greater numbers of observations.

4. ANALYSIS

4.1 MATERNAL BEHAVIOUR

This part of the study estimates time budgets for a variety of maternal behaviours. These estimates are calculated from the total percentage of time in a focal video (POF) spent performing the behaviour. The two focal video types used (nursing and random) represent different aspects of mother-pup relationships (section 3.1.3). Nursing focal videos capture a less restful time period, specifically timed around nursing bouts. These represent periods of more directed mother-pup interactions, focussed on patterns of nursing behaviour. Random focal videos, on the other hand, aimed to investigate non-nursing related behaviour, a focal video was only considered 'random' if no nursing occurred throughout its length. Throughout this chapter, sample size (n) of a category (e.g. early lactation; late lactation; mothers with male pups; mothers with female pups) refers to both the number of focal videos used and the number of individually known females included in that category (only one video per known female was included in each category).

This chapter assesses individual variation in broad categories of maternal behaviour (defined in section 3.3) and identifies any evidence of consistent individual differences (CIDs). Selected behaviours are play between mother and pup (Play), all mother-pup interaction both positive and negative but without nursing (PINT), and vigilance (split into alert and pup-check). Vigilance measures are separated into POF (also called duration) and rate per minute. From nursing focal videos, POF spent on nursing is analysed, as is POF spent on PINT including nursing. Pup-check counts and rates made *in situ* (in-field pup-check counts) have been included. Prior to examining the existence of CIDs, the effect of the lactation stage on the expression of these behaviours is assessed. Evidence of CIDs is then assessed. Finally, potential reasons for varied maternal attendance (given in section 2.1.3) are assessed for their impact on maternal behaviour as an additional explanation for the variation seen in **Figure 4.1.1**.

4.1.1 General patterns of behaviour

As discussed in section 2.1.3.2, grey seal mothers rely on limited resources, sequestered primarily as blubber prior to the breeding season, to support both their own energetic requirements and to supply nutrition to their pups. For this reason, females on breeding colonies spend a vast majority of time resting. The breeding colony at Donna Nook is no exception. During this study, females were observed to spend an average of 49.4% of nursing focal videos resting, increasing to 69.4% during random focal videos. The remainder of their

time is spent performing solitary behaviours (those which did not involve interaction with any other individual; including comfort movements and time spent drinking), moving around the colony, interacting with other adults (both aggressively and sexually), performing vigilance behaviours, and interacting with their pups.

Excluding nursing, time spent interacting with pups (PINT) averaged between 9-10% of both nursing and random focal videos. Some mothers were observed 'playing' with pups, including nosing, rolling and play-snap behaviours. These were generally of short duration in both nursing and random focal videos, accounting for an average POF of 3.03% and 1.63% respectively. However, time given to these play behaviours was highly variable between mothers. For example, the highest POF spent playing was recorded for mother P36, who spent 20.5% of one nursing focal playing with her pup. Some mothers were consistently recorded as spending 0% of focal videos playing. This is indeed the case for mother P26 for whom three random focal videos were recorded and mothers R12 and R5, both of whom had two random focal videos. No play behaviours were observed during any of these seven focal videos. However, play behaviours occurred infrequently throughout the day, and the likelihood of a single 30 minute focal capturing these behaviours in a way which was reflective of the true time budget of a mother and pup is unlikely. This variation is true also of PINT behaviours. The smallest percentage of a nursing focal spent performing PINT behaviours was 0.83% for mother RS3M. By comparison, the highest POF was 31.56% for mother P7.

Vigilance behaviours were of particular interest during this project, having been identified as being repeatable in earlier studies (Twiss & Franklin, 2010; Twiss *et al*., 2011; Twiss *et al*., 2012). Vigilance behaviours include both alert behaviours and pup-checks made by the mother. In this study, alert behaviours accounted for an average POF of 7.8% of all nursing and random focal videos, while pup-checks took an average POF of 3.4%. The POF spent performing alerts and pup-checks varied between focal videos. For alert behaviours, POF of each individual focal showed a range of 0.7% to 20.41%; for pup-checks the range was 0.53% to 10.38% of each individual focal. The box-plots in **Figure 4.1.1** show the median POF spent on behaviours, and the variation of these across all mothers with focal videos. Results have been split between early and late lactation and separate graphs are given for nursing focal videos and random focal videos.

Table 4.1.1 provides colour and pattern coding used for box-plots throughout this analysis. Lactation stage, pup gender and site are generally not displayed on the same plot. There is one exception to this. The random focal video plot, comparing public and RAF sites for play and alert duration

behaviours (**Figure 4.3.5**). It was necessary to separate these behaviours into early and late lactation for comparison, as there were significant differences between lactation stages. Lactation stage is indicated on the horizontal axis for this plot.

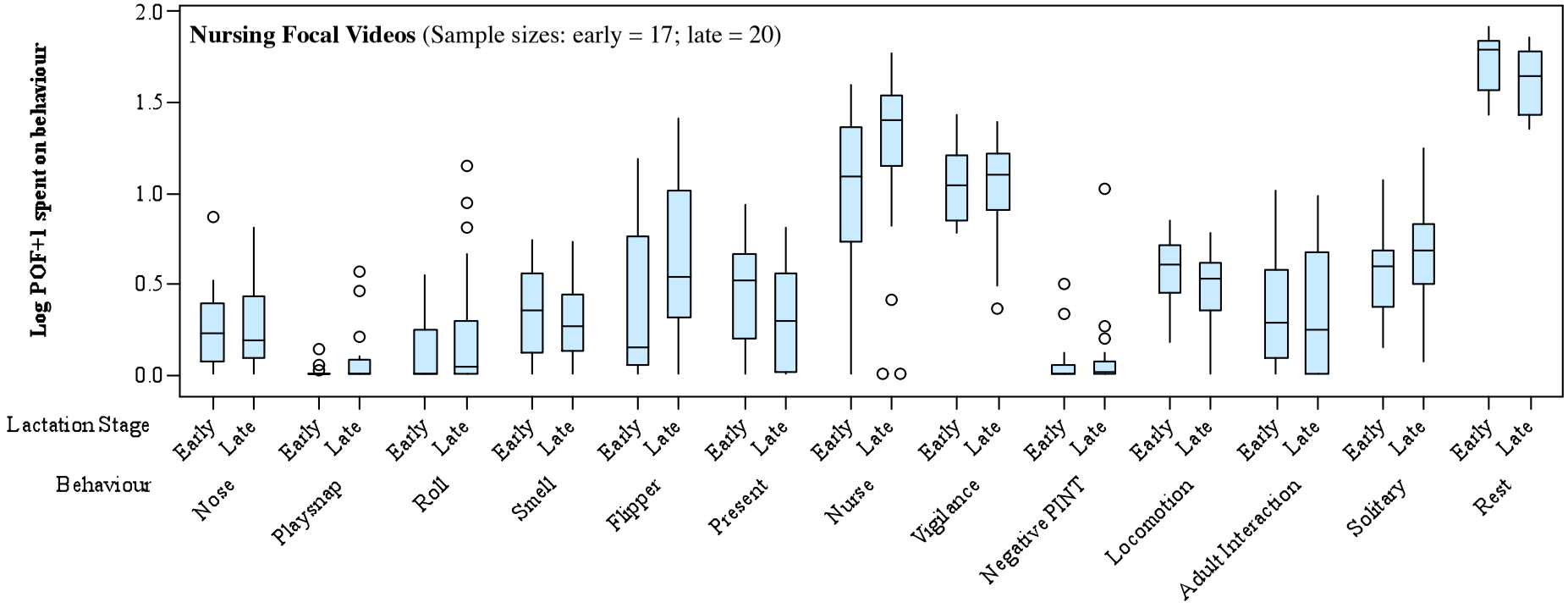


Figure 4.1.1a POF of nursing focal videos (shown on log base 10 scale using POF + 1) spent performing various behaviours. These are separated into early and late lactation for each behaviour. Some behaviours are grouped (vigilance: alert behaviours and pup-checks; negative PINT: howl, hiss, snap and reject pup; adult interaction: aggression and copulation; solitary: comfort move and drinking). O represents an outlier.

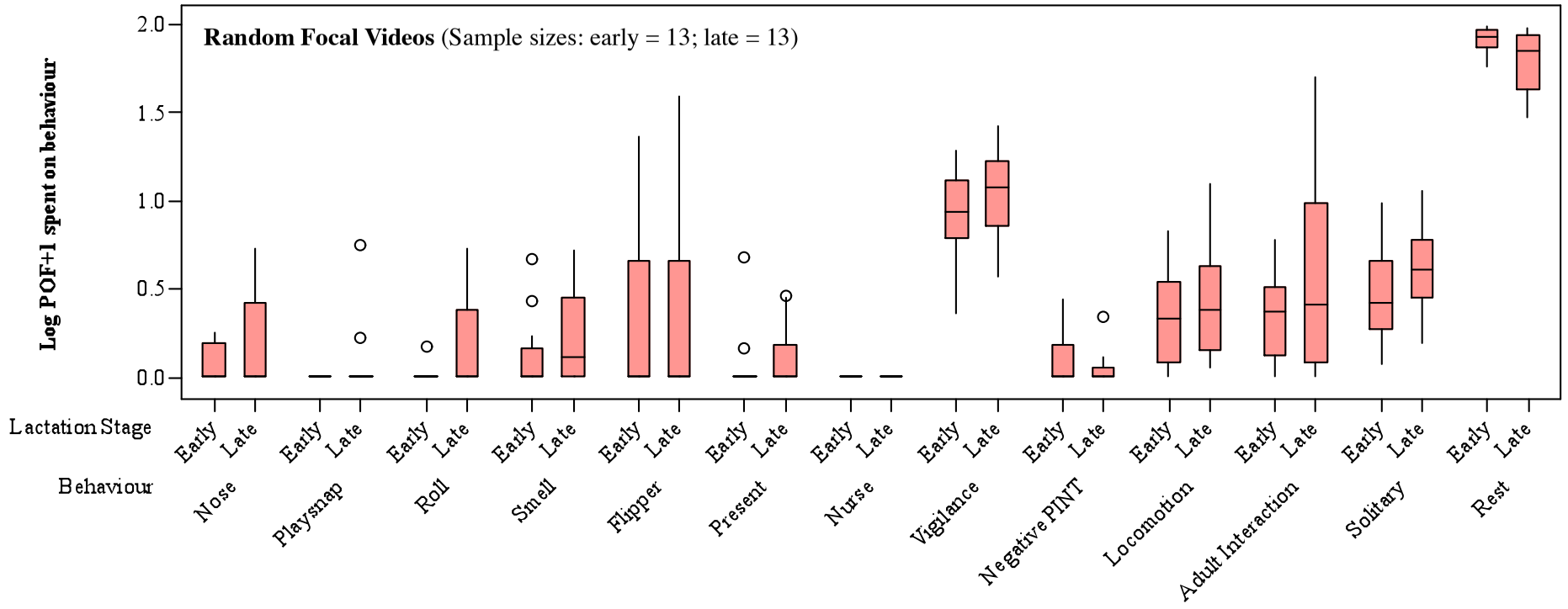


Figure 4.1.1b POF of random focal videos (shown on log base 10 scale using POF + 1) spent performing various behaviours. These are separated into early and late lactation for each behaviour. Some behaviours are grouped (vigilance: alert behaviours and pup-checks; negative PINT: howl, hiss, snap and reject pup; adult interaction: aggression and copulation; solitary: comfort move and drinking). O represents an outlier.

4.2 IDENTIFYING MOTHERING STYLES

4.2.1 Differences in maternal time budgets across lactation stages

The following analyses address question 1. set out in section 2.2.4: Does the proportion of time spent on certain maternal behaviours change between early lactation and late lactation?

A two-tailed randomisation test (Todman & Dugard, 2001) was used to identify differences in maternal behaviours between early and late lactation. Analysis of nursing focal videos show a significant difference in the average POF spent nursing between early and late lactation (**Table 4.2.2**). Nursing constituted a greater percentage of late lactation nursing focal videos than those during early lactation. Similarly, mother-pup interaction including nursing accounted for a greater POF during late lactation focal videos than early. This difference is more significant than when nursing is considered alone. This suggests that, within individual focal videos, PINT and nursing add up in such a way that the difference between early and late lactation is exacerbated. The difference in mean POF spent on PINT between early and late lactation is approximately 2%, in nursing it is approximately 10%. This difference increases to 16% when PINT and nursing are combined. Throughout random focal videos, there appeared to be a trend for a greater POF spent playing during late lactation. This was not statistically significant, however. There is no statistical evidence that maternal time budgets, for the other behaviours considered, differ between early and late lactation. This is reflected both by the statistical analysis (**Table 4.2.2**), and by similarities in median values and ranges, shown by the box-plots in **Figures 4.2.1** to **4.2.3**.

In nursing focal videos, POF spent on PINT, nursing and PINT including nursing show high levels of variation about the median (**Figure 4.2.1**). So, too, do pup-check rates both from focal videos and in-field counts. This suggests the possibility of variation between individuals in these behaviours, which is further supported by the results of the ICC (**Table 4.2.3**). Alert duration also shows large variation about the median (**Figures 4.2.1** and **4.2.2**), however this is not explained by CIDs. Instead, there appears to be variation within individuals. This is reflected by the coefficients of variation (CV), calculated for each female individually (**Table 4.2.1**). The CV represents the ratio of standard deviation of the mean, to the mean. Each female was analysed separately, measuring the duration of each alert bout performed, from all focal videos available. For each female included in the focal video study, the CV was above 0.65; i.e. the standard deviation of each individual's alert duration represented at least 65% of the average. This suggests that each cow showed large variation in the duration of each occurence of an alert behaviour.

Table 4.1.1 Colour coding for boxplots throughout the analysis (excluding section 4.3.2.2) unless otherwise indicated on graph axes

Colour Key		Pattern Key			
Nursing Focal		Early Lactation		Late Lactation	
Random Focal		Male Pup		Female Pup	
In-field Count		Public Site		RAF Site	

Table 4.2.1 CV in the duration of bouts of alert behaviours for each female.

Seal ID	CV	Seal ID	CV	Seal ID	CV	Seal ID	CV
R21	0.67	**R16**	0.81	**P29**	0.86	**R12**	0.99
P7	0.68	**R9**	0.81	**P36**	0.87	**P15**	1.01
P26	0.74	**R25**	0.83	**R14**	0.87	**R20**	1.07
P37	0.76	**R13**	0.84	**P30**	0.89	**R17**	1.09
P20	0.77	**P16**	0.85	**R26**	0.92	**R5**	1.11
RS3M	0.78	**P21**	0.85	**R11**	0.94	**LINDA**	1.15
P22	0.79	**R1**	0.85	**R7**	0.96		

Table 4.2.2 Outcomes of two-tailed randomisation tests (Todman & Dugard, 2001) comparing the percentage of time during focal videos spent performing behaviours or the rate of these behaviours per minute between early and late lactation. 'n' denotes sample size. In field pup-check rates were compared using a Mann-Whitney U test.

Behaviour	**Early Lactation**		**Late Lactation**		**Randomisation**				**Figure**
	Mean	S.E	Mean	S.E	Statistic	**p-value**	Significance		
							0.05	0.1	
Nursing Focal	**n = 17**		**n = 20**						
Play	1.66	0.52	3.13	1.16	1.47	**0.33**	x		**4.2.1**
PINT	8.71	1.56	10.97	2.03	2.26	**0.40**	x		**4.2.1**
PINT incl. nursing	19.48	3.03	34.98	3.62	15.5	**0.003**	✓		**4.2.1**
Nursing	14.25	2.81	24.01	3.67	9.76	**0.05**	✓		**4.2.1**
Alert Duration	7.80	1.06	7.78	1.02	0.02	**0.99**	x		**4.2.1**
Alert Rate	1.17	0.18	1.12	0.15	0.05	**0.85**	x		**4.2.3**
Pup-check Duration	4.08	0.66	2.99	0.39	1.09	**0.15**	x		**4.2.1**
Pup-check Rate	1.01	0.14	0.84	0.10	0.17	**0.29**	x		**4.2.3**
Random Focal	**n = 13**		**n = 13**						
Play	0.26	0.10	2.06	0.93	1.8	**0.07**	x	✓	**4.2.2**
PINT	4.15	1.84	9.56	4.25	5.41	**0.26**	x		**4.2.2**
Alert Duration	5.81	1.06	7.97	1.44	2.16	**0.23**	x		**4.2.2**
Alert Rate	1.15	0.16	2.72	0.39	0.33	**0.16**	x		**4.2.3**
Pup-check Duration	2.72	0.39	3.55	0.56	0.83	**0.25**	x		**4.2.2**
Pup-check Rate	0.73	0.11	0.86	0.12	0.13	**0.43**	x		**4.2.3**

Behaviour	**Early Lactation Median**	**Late Lactation Median**	**Mann-Whitney U**			**Figure**
			W value	**p-value**	Significance	
In-field Counts	**n = 38**	**n = 38**				
Pup-check rate /m^{-1}	0.57	0.50	1588.5	**0.19**	x	

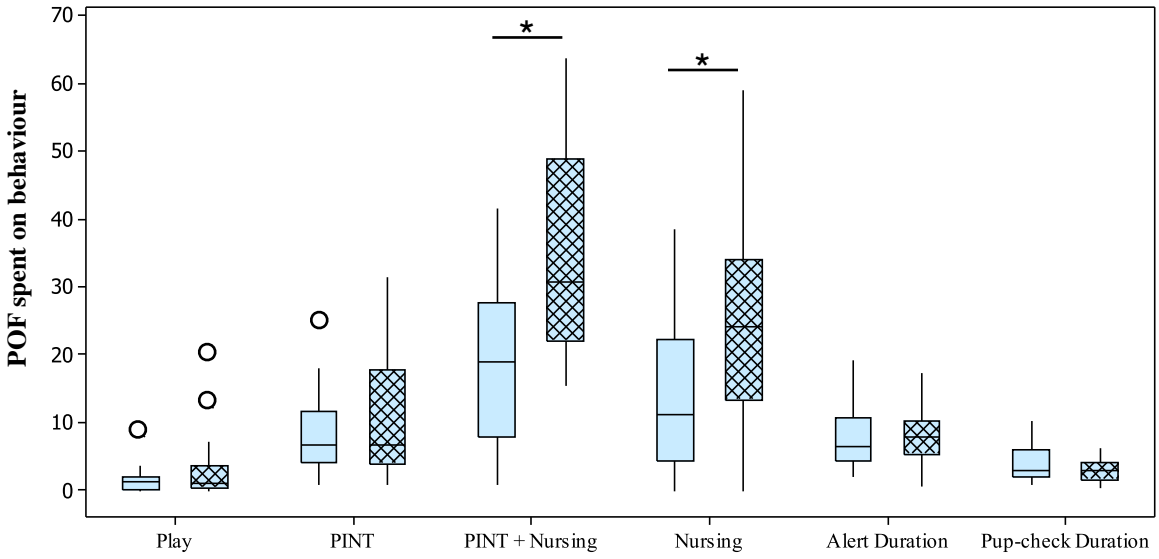


Figure 4.2.1 POF of nursing focal videos spent on broad category behaviours compared between early (clear bars) and late (hashed bars) lactation. O represents an outlier. * represents significance to the 0.05 level.

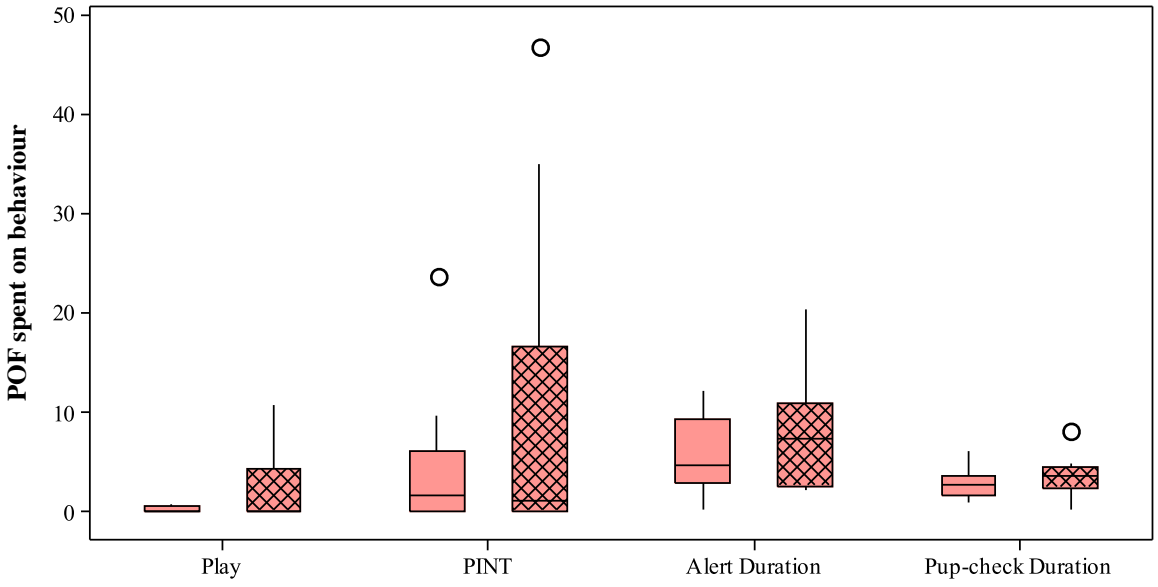


Figure 4.2.2 POF of random focal videos spent on broad category behaviours compared between early (clear bars) and late (hashed bars) lactation. O represents an outlier.

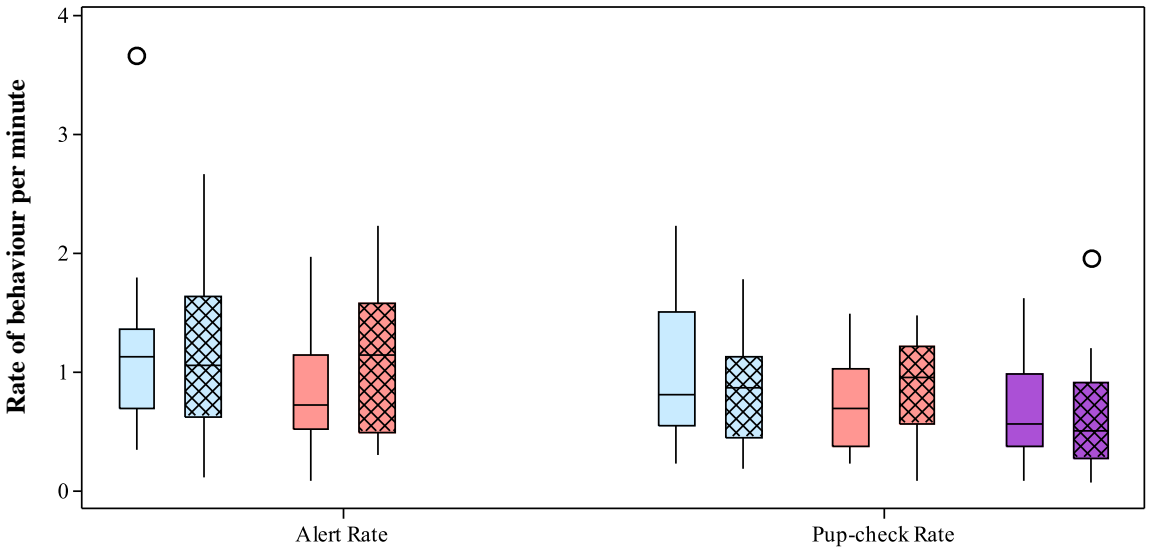


Figure 4.2.3 Rate per minute of alert and pup-check behaviours, compared between early (clear bars) and late (hashed bars) lactation. Rates taken during nursing (blue) and random (red) focal videos spent as well as pup-check rate per minute calculated from in-field counts (purple). O represents an outlier.

4.2.2 Focal Results

The following analyses address part 'a' of question 1, set out in section 2.2.4: Are there consistent individual differences (CIDs) in behaviour between mothers, when compared between early lactation and late lactation.

The intraclass correlation coefficient (ICC) was used to assess the existence (if any) of CIDs amongst maternal behaviours. Categories of maternal behaviours assessed are listed with their ICC number, test statistic and p value in **Table 4.2.3**. ICC calculations using nursing focal videos compare data between early and late lactation. Mothers with both early and late random focal videos were not so numerous, as random focal videos often developed into nursing focal videos, when the mother began nursing her pup. When this occurred, a random focal was not taken for that day. In order to increase the size of the data set, when using the ICC with random focal videos, any mother with two random focal videos was included regardless of which period of lactation these were taken during.

Results from nursing focal videos suggest CIDs in the time spent on non-nursing PINT (ICC = 0.44, $F_{14,14}$ = 2.4, p = 0.05; **Figure 4.2.4***a*) as well as nursing (ICC = 0.42, $F_{14,14}$ = 3.2, p = 0.02; **Figure 4.2.4***b*). However, the former is vulnerable to the results of mother P7. The significance of the CIDs increases when PINT and nursing behaviours are considered together (ICC = 0.57, $F_{14,14}$ = 5.5, p = 0.02; **Figure 4.2.5***a*). The results suggest the possibility of CIDs with respect to the rate of alert behaviours per minute, however this was not significant to the 0.05 level (ICC = 0.41, $F_{14,14}$ = 2.3, p = 0.07; **Figure 4.2.5***b*). This trend did not extend to the POF spent performing alert behaviours (alert duration).

During random focal videos, there appeared to be a trend toward CIDs in both play (ICC = 0.37, $F_{9,9}$ = 2.4, p = 0.1; **Figure 4.2.6***a*) and pup-check rates (ICC = 0.49, $F_{9,9}$ = 2.9, p = 0.07; **Figure 4.2.6***b*), however these results were not significant. **Figure 4.2.6***a* shows a number of females (P26, R5, R12 and R13) clustered in the lower left corner, with POF spent on play recorded at 0% (or just over) for both early and late lactation. It is likely the consistency of not performing play behaviours, shown by almost all females in this analysis, that the ICC result is reflecting.

4.2.3 Pup-Check Count Results

The following analyses address part 'a' of question 1, set out in section 2.2.4: Are there consistent individual differences (CIDs) in behaviour between mothers, when compared between early lactation and late lactation.

Early and late pup-check counts were collected from a total of twenty-six females. Pup-check counts have previously been shown to be repeatable in grey seals. In this study, pup-check counts or rates per minute from focal videos did not show evidence of CIDs (**Table 4.2.3**). However, pup-check

counts taken in the field do appear to. The number of pup-checks made in the space of 15 minutes was found to be highly repeatable across 15 females (ICC = 0.21, $F_{25,125} = 2.8$, p = 0.0001; **Figure 4.2.7***a*). In this case, the ICC compared six counts per female, three from early lactation and three from late. Pup-check rates per minutes, calculated from the combined counts from each lactation stage, were also found to be repeatable (ICC = 0.28, $F_{25,25} = 2.1$, p = 0.03; **Figure 4.2.7***b*).

Table 4.2.3 ICC statistics for identification of CIDs in maternal behaviour. n denotes sample size. Degrees of freedom represented by subscript beside F value.

Behaviour	ICC	F value d.f.	p-value	Confidence intervals		Significance		Figure
				Lower	Upper	0.05	0.1	
Nursing Focal Videos: ICC compares early lactation with late lactation (n = 15)								
Play	0.08	$1.2_{14,14}$	**0.39**	-0.47	0.56	x		
PINT	0.44	$2.4_{14,14}$	**0.05**	-0.104	0.77	✓		**4.2.4***a*
PINT incl. nursing	0.57	$5.5_{14,14}$	**0.002**	0.025	0.84	✓		**4.2.5***a*
Nursing	0.42	$3.2_{14,14}$	**0.02**	-0.051	0.75	✓		**4.2.4***b*
Alert Duration	0.01	$1.0_{14,14}$	**0.49**	-0.49	0.50	x		
Alert Rate	0.41	$2.3_{14,14}$	**0.07**	-0.14	0.75	x	✓	**4.2.5***b*
Pup-check Duration	0.25	$1.8_{14,14}$	**0.15**	-0.20	0.65	x		
Pup-check Rate	0.26	$1.8_{14,14}$	**0.14**	-0.20	0.66	x		
Random Focal Videos: ICC compares first focal with second focal (n = 9)								
Play	0.37	$2.4_{9,9}$	**0.10**	-0.18	0.78	x	✓	**4.2.6***a*
PINT	0.09	$1.2_{9,9}$	**0.40**	-0.49	0.64	x		
Alert Duration	0.15	$1.3_{9,9}$	**0.34**	-0.58	0.72	x		
Alert Rate	0.27	$1.7_{9,9}$	**0.23**	-0.41	0.77	x		
Pup-check Duration	0.28	$1.8_{9,9}$	**0.21**	-0.36	0.77	x		
Pup-check Rate	0.49	$2.9_{9,9}$	**0.07**	-0.16	0.85	x	✓	**4.2.6***b*
In-field Counts: ICC compares early lactation with late lactation (n = 15)								
No. pup-checks in 15 mins	0.21	$2.8_{25,125}$	**0.0001**	0.08	0.40	✓		**4.2.7***a*
Pup-check rate / m^{-1}	0.28	$2.1_{25,25}$	**0.03**	-0.03	0.61	✓		**4.2.7***b*

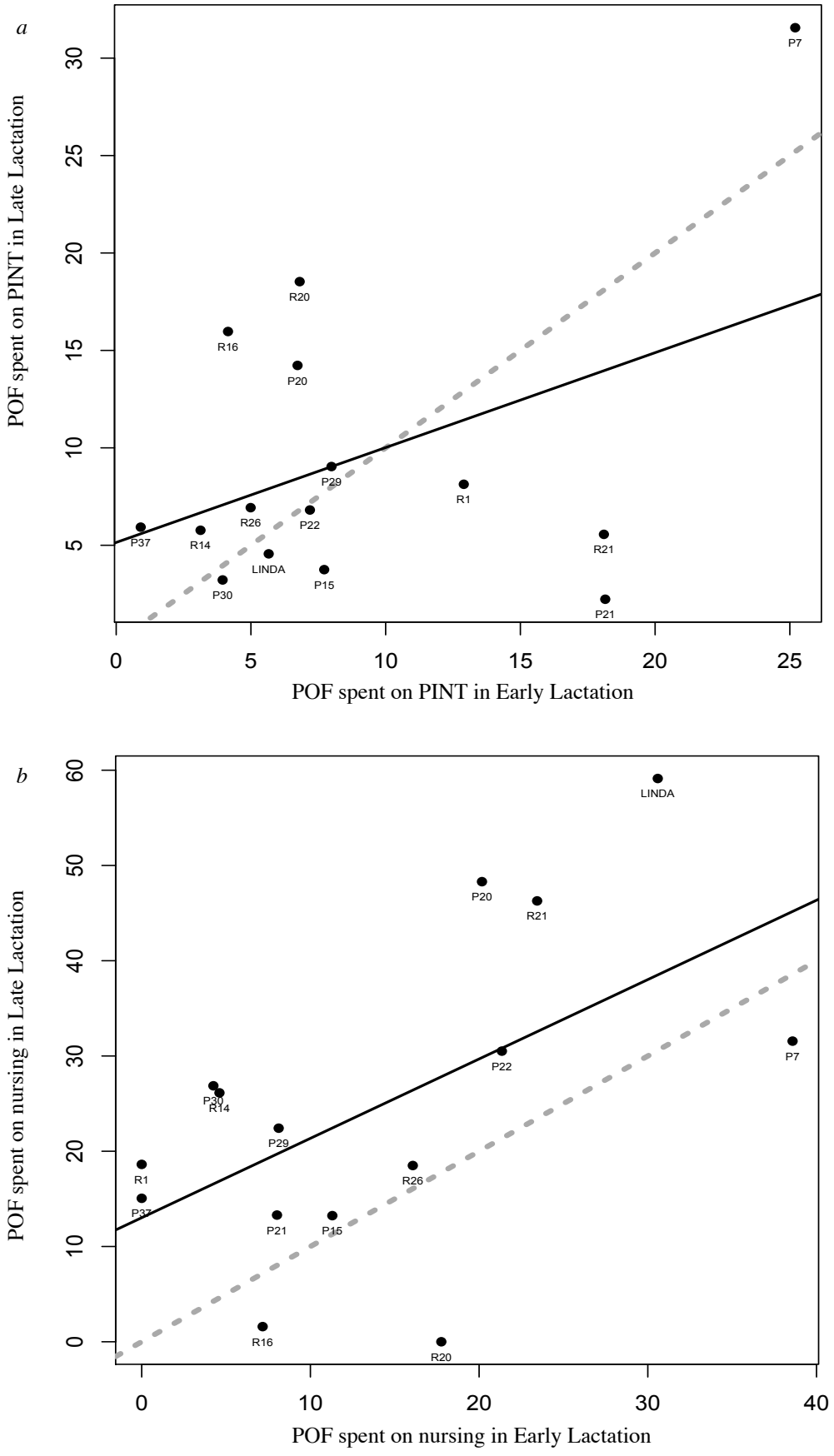


Figure 4.2.4 POF in nursing focal videos spent on *a*. PINT; *b*. nursing; Each point represents an individually known female. Solid lines represent lines of best fit; dotted lines represent 1:1 line.

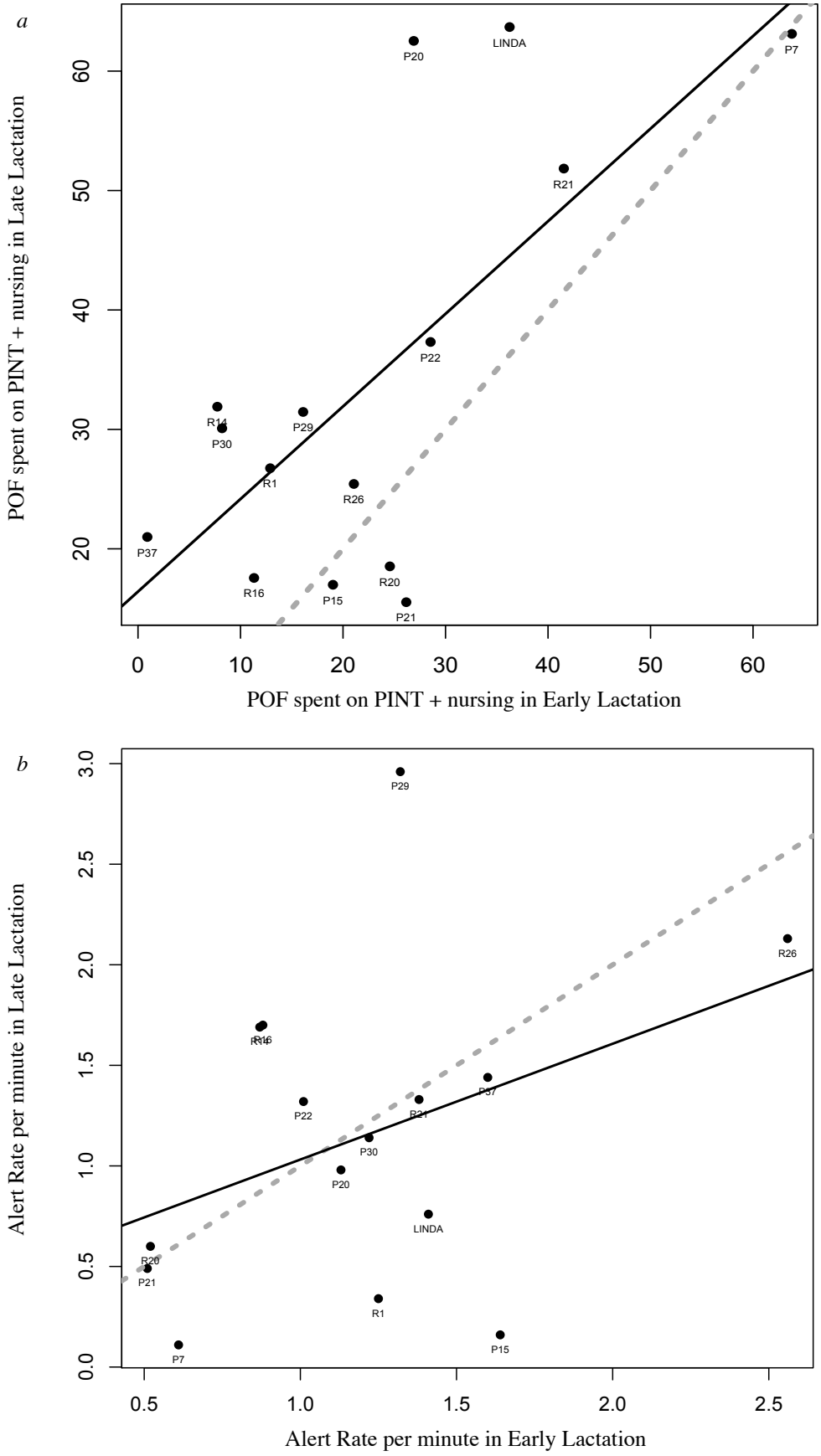


Figure 4.2.5 *a*. POF in nursing focal videos spent on PINT including nursing; *b*. rate per minute of alert behaviours in nursing focal videos. Each point represents an individually known female. Solid lines represent lines of best fit; dotted lines represent 1:1 line.

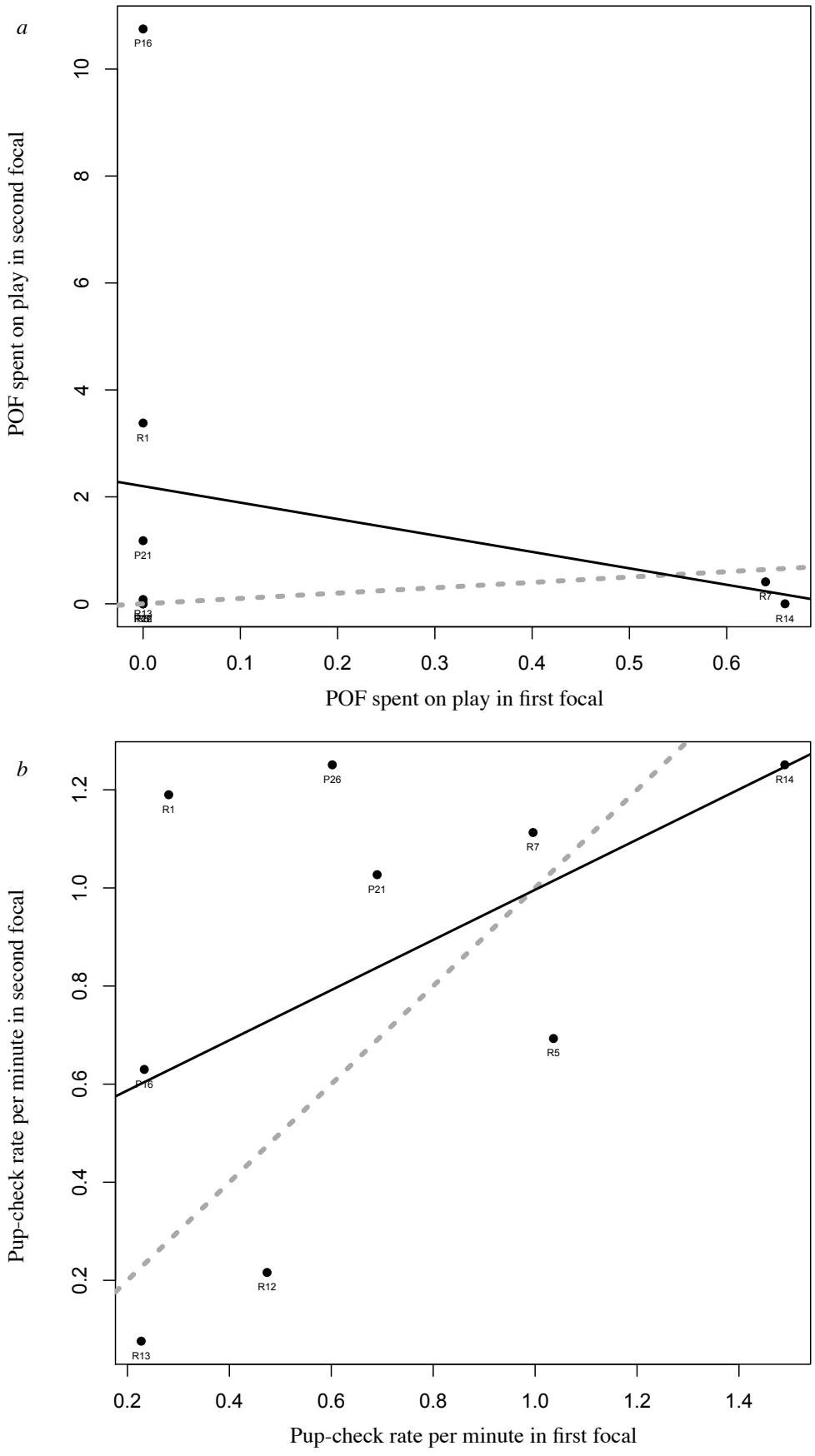


Figure 4.2.6 Comparison between first and second random focal videos for *a*. POF in random focal videos spent on play *b*. Rate per minute of pup-check behaviours. Each point represents an individually known female. Solid lines represent lines of best fit; dotted lines represent 1:1 line.

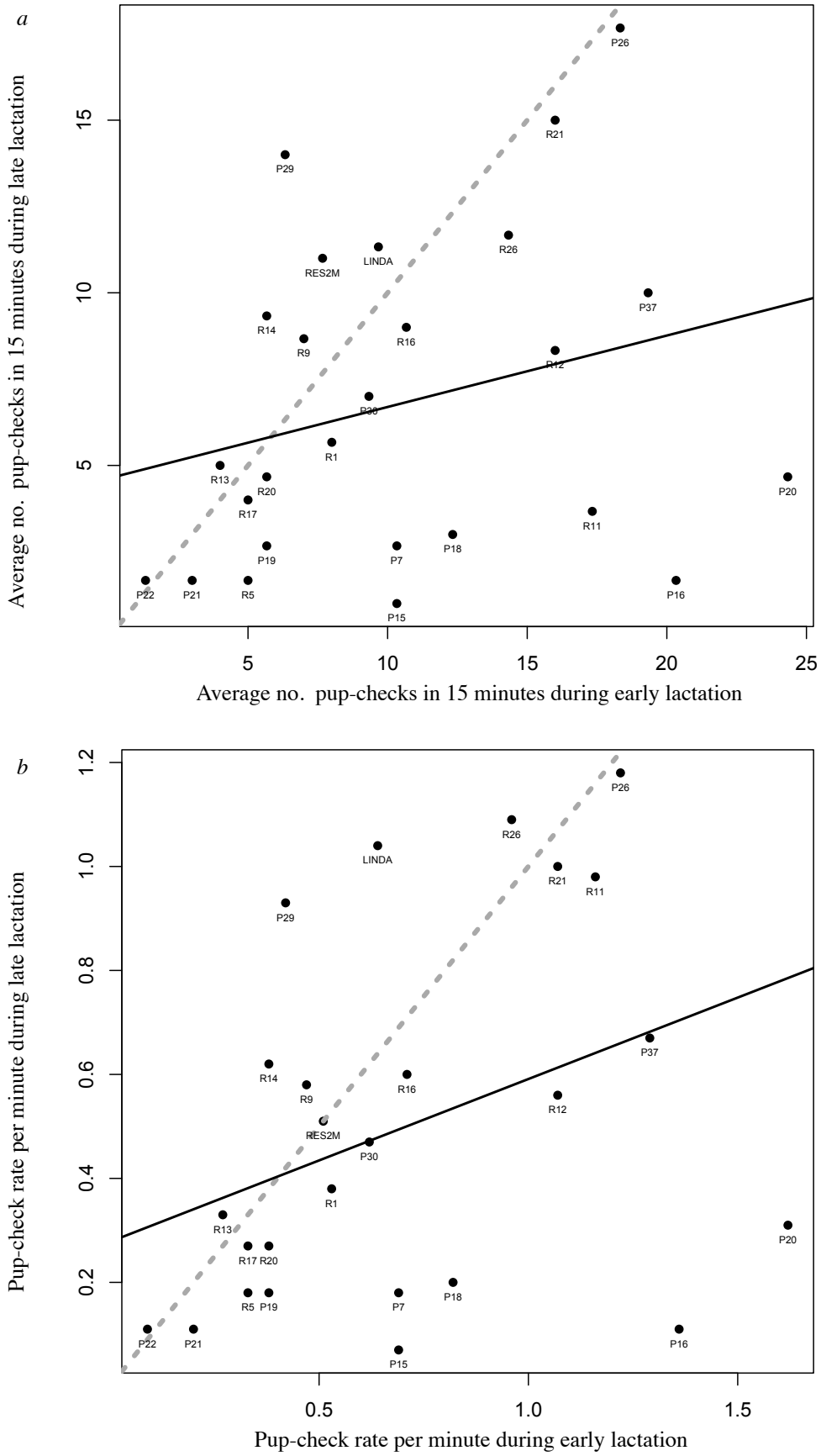


Figure 4.2.7 In-field pup-check counts, comparing early to late lactation for *a*. average number of pup-checks made in 15 minutes; *b*. Pup-check rate per minute. Each point represents an individually known female. Solid lines represent lines of best fit; dotted lines represent 1:1 line.

4.2.4 Mother-Offspring Relationship Quality (MORQ) Index

The following analyses address part 'b' of question 1, set out in section 2.2.4: Are some mothers consistently more affiliative than others?.

The Mother-Offspring Relationship Quality (MORQ) index was adapted from that used by Weaver & de Waal (2002). Weaver & de Waal used relative affiliation to relative agonism ratios calculated from the mother-offspring (MO) relationship. Preliminary analysis of MO relationships in grey seals suggested that mothers spent a negligible amount of time acting agonistically towards their own pup (see negative PINT in **Figure 4.1.1**). Instead, rejective behaviour appeared to provide a better comparison. There was variation between focal videos in the time spent resting, performing solitary behaviour, interacting with other individuals and moving around the colony. These behaviours are independent of positive PINT (no interaction with the pup was observed in conjunction with any of these behaviours throughout the current study), therefore for the purpose of this study the MORQ index compares the time spent on affiliative behaviours (positive PINT and nursing) with time spent on agonistic behaviours (negative PINT) as well as non-PINT-behaviours.

The MORQ index split females at Donna Nook into two very distinct groups. Eleven females received a MORQ index value of under 1.5 both during early lactation and late lactation. This suggests that, relative to other females with pups of the same age, these mothers show particularly high levels of negative PINT and/or non-PINT orientated behaviours. **Table 4.2.4** shows the results of the ICC conducted using MORQ index values, both for nursing and random focal videos. Four females scored MORQ index values of over 1.5 for either early or late

Table 4.2.4 ICC statistics for identification of CIDs in mother-offspring relationship quality (MORQ) index. n denotes sample size. Degrees of freedom represented by subscript beside F value.

Behaviour	ICC	F value d.f.	p-value	Confidence intervals		Significance		Figure
				Lower	Upper	0.05	0.1	
Nursing Focal Videos: ICC compares early lactation with late lactation (n = 15)								
MORQ	0.65	$4.5_{14,14}$	**0.004**	0.22	0.87	✓		**4.2.8***a*
Random Focal Videos: ICC compares first focal with second focal (n = 9)								
MORQ	-0.30	$0.59_{8,8}$	**0.77**	-0.95	0.47	x		**4.2.8***b*

lactation, and two scored over 1.5 for both. These are the mothers which show unusually high levels of positive PINT and nursing behaviours relative to other mothers. The ICC calculation suggests the presence of CIDs in the MORQ index values around nursing bouts (ICC = 0.65, $F_{14,14}$ = 4.5, p = 0.004). **Figure 4.2.8***a* clearly shows the divide between the eleven rejective or 'laissez-faire' mothers and the four more affiliative mothers.

This is not the case with MORQ index calculations made from random focal videos. The ICC value for random focal videos suggest there is no repeatability between first and second focal MORQ index values. This is displayed in **Figure 4.2.8***b*, where points are spread along the extremities of the axes and do not form a clear line.

The more attentive mothers did not appear to check their pup more often. Comparisons between MORQ index values and in-field pup-check rates using Spearman's Rank correlation showed no correlation (early lactation: r = 0.1, n = 15, p = 0.73 **Figure 4.2.9***a* red; late lactation: r = 0.4, n = 15, p = 0.17 **Figure 4.2.9***a* green). Affiliation levels did not appear to be linked to pupping earlier or later in the season, as there was no significant correlation (again using Spearman's Rank) between MORQ values and estimated pupping date relative to others in the study (r = 0.1, n = 15, p = 0.66 **Figure 4.2.9***b*).

Finally, more affiliative mothers did not retain their pups at a close proximity on a regular basis. Analysis with Todman & Dugard's 2001 design 5 found no significant link between MORQ index values and mother-pup proximity (early lactation: randomisation statistic = 14.42, p = 0.22; late lactation: randomisation statistic = 13.59, p = 0.39). Figures **4.2.10** and **4.2.11** show the number of occasions on which mother and pup were within 1bl of each other compared to the range of MORQ values of mothers in these categories. These figures represent early and late lactation respectively.

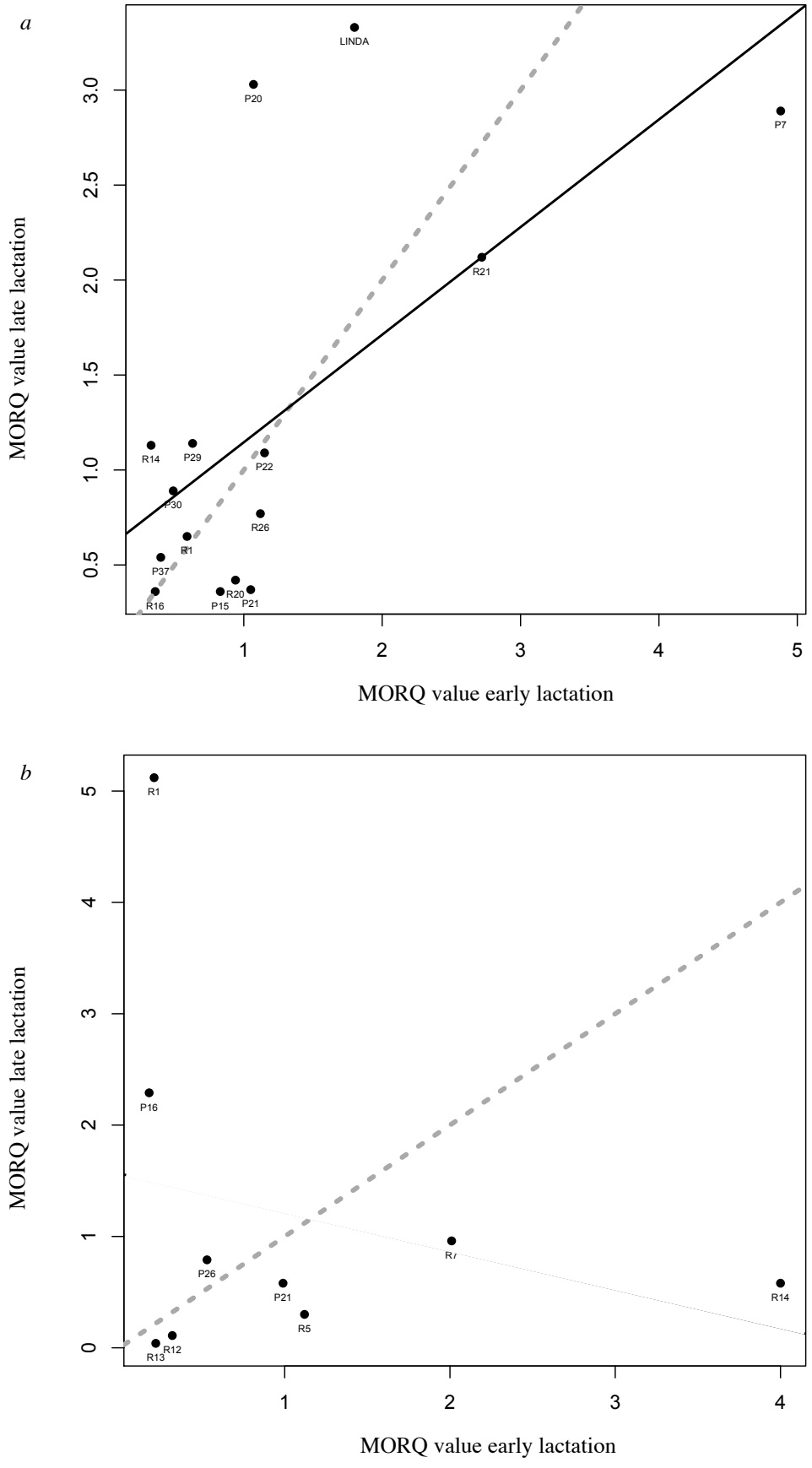


Figure 4.2.8 *a*. MORQ index values from nursing focal videos; *b*. MORQ index values from random focal videos. Each point represents an individually known female. Solid lines represent lines of best fit; dotted lines represent 1:1 line.

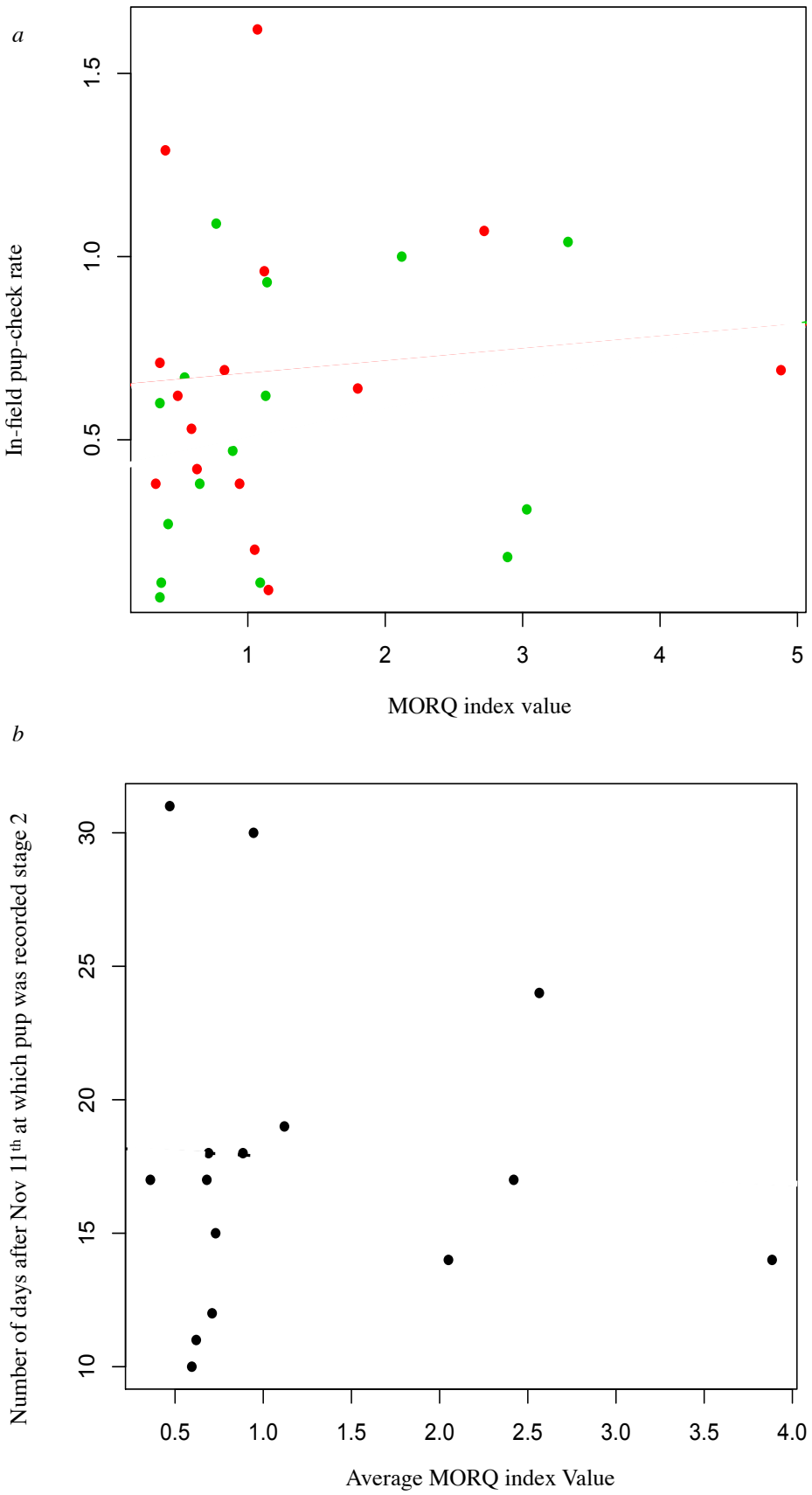


Figure 4.2.9 *a*. MORQ index value against rate per minute of pup-checks from in-field counts. Red represents early lactation, green late lactation; *b*. average MORQ index value against relative birth date. Each point represents an individually known female. Solid lines represent lines of best fit.

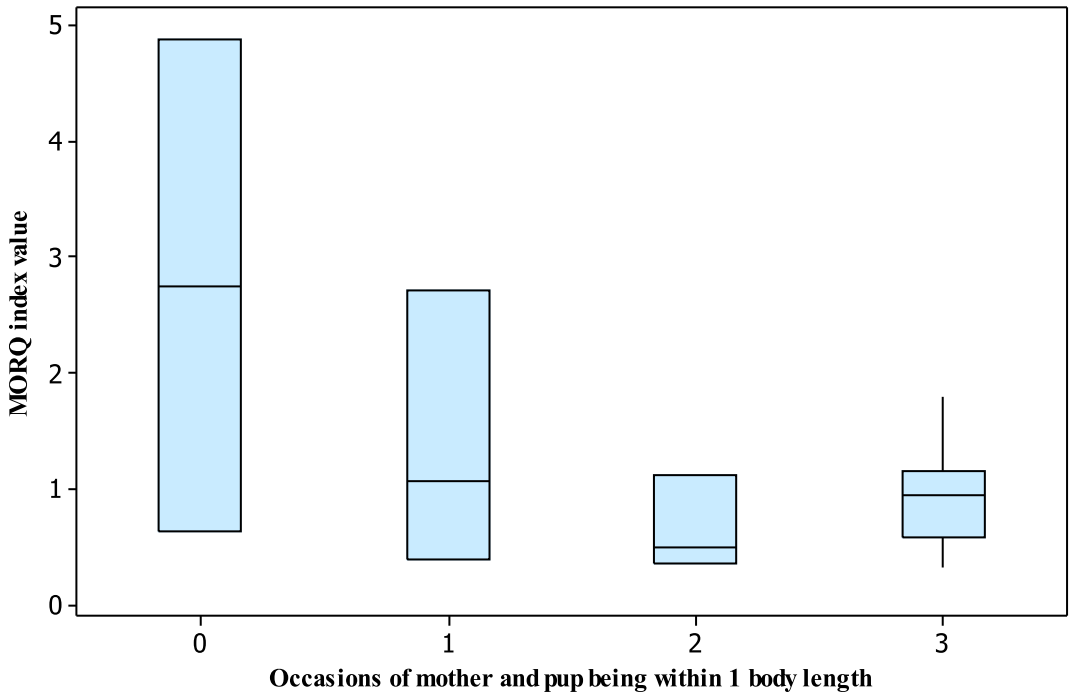


Figure 4.2.10 MORQ index values of mothers, set against the number of proximity maps (out of 3) in which the mother was recorded within 1bl of her pup. MORQ index values and proximity measures from early lactation. Sample sizes: 0 Occasions = 2; 1 occasion = 3; 2 occasions = 3; 3 occasions = 7.

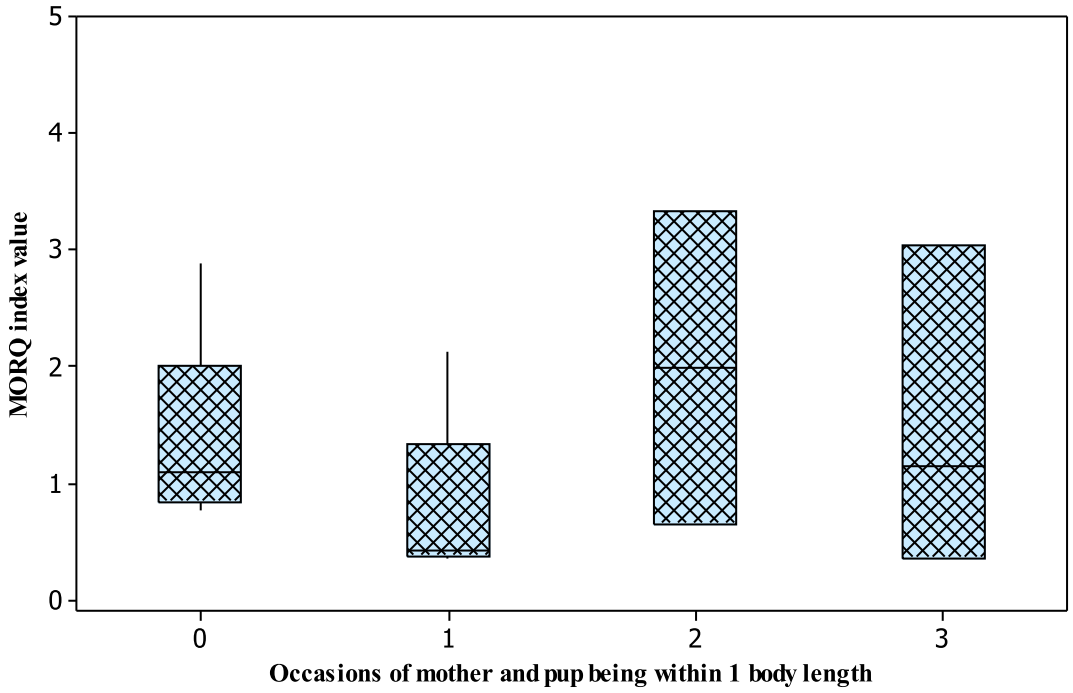


Figure 4.2.11 MORQ index values of mothers, set against the number of proximity maps (out of 3) in which the mother was recorded within 1bl of her pup. MORQ index values and proximity measures from late lactation. Sample sizes: 0 Occasions = 5; 1 occasion = 5; 2 occasions = 2; 3 occasions = 3.

4.3 POSSIBLE DRIVERS OF MATERNAL BEHAVIOUR

As discussed in section 2.1.3 there are a number of factors which may influence maternal behaviour. Two factors were selected for analysis in the current study: pup gender and environmental disturbance, (split into regular and sudden disturbance).

4.3.1 Pup Gender

The following analyses address question 2, set out in section 2.2.4: Does the gender of the pup influence maternal time budgets?

Pup gender was discriminated in the field. This was not possible for all pups of identified females. Gender is difficult to identify in earlier stage pups (stages 1 and 2). To successfully discern gender, ventral surface of the pup must be visible to the observer. However, in this study the observer was stationary and thus unable to move around the colony. In addition, due to the nature of the substrate on which the colony settled, the ventral fur of the pups was often obscured with a covering of sand, silt or mud. To avoid misrepresentation, only females with pups whose gender could be identified with confidence were included in this comparison. Consequently, sample sizes for data extracted from focal videos in this section are small (indicated in **Table 4.3.1**). With this in mind, the results from this section of the analysis must be considered with caution, representing a small proportion of the population, though they do suggest no great difference between mothers of male pups and those of female pups. **Table 4.3.1** shows a comparison of the main maternal behaviours using a two-tailed randomisation test from Todman & Dugard (2001). The results suggest that pup gender at Donna Nook had no significant impact on the level of maternal attentiveness afforded to offspring. There did appear to be a trend towards mothers of female pups spending, on average, more time performing alert behaviours, at a higher rate per minute, however this was not significant at the 0.05 level. The box-plots in **Figure 4.3.1** and those in blue in **Figure 4.3.3** show behavioural data collated from nursing focal videos. These show clearly the trend described for alert duration and rate respectively. They also show similarities in the interquartile ranges for mothers with male and female pups for PINT, pup-check duration and pup-check rate. When considering random focal behaviour (**Figure 4.3.2** and red boxes in **Figure 4.3.3**) mothers of female pups seem to range more in time spent on play and PINT, and in the number of pup-checks and alert behaviours per minute. Pup-check rates from in-field pup-check counts were calculated and comparison made between mothers of male and female pups. Sample sizes were slightly larger in this data set, with ten mothers having male pups and eight with female pups. Again no significant difference was found between the mean rates of pup-checks of mothers with male pups and mothers with female pups.

Table 4.3.1 Outcomes of two-tailed randomisation tests (Todman & Dugard, 2001) comparing the percentage of time during focal videos spent performing behaviours or the rate of these behaviours per minute between mothers with female pups and those with male pups. 'n' denotes sample size.

Behaviour	**Male pups**		**Female pups**		**Randomisation**				**Figure**
	Mean	S.E	Mean	S.E	Statistic	**p-value**	Significance		
							0.05	0.1	
Nursing Focal	**n = 8**		**n = 4**						
Play	3.97	1.81	5.7	4.94	1.73	**0.68**	x		**4.3.1**
PINT	11.77	3.79	11.26	4.52	0.5	**0.95**	x		**4.3.1**
PINT incl. nursing	39.99	7.63	33.56	3.57	6.44	**0.56**	x		**4.3.1**
Nursing	28.22	7.02	22.29	6.65	5.93	**0.62**	x		**4.3.1**
Alert Duration	7.10	1.42	11.23	1.18	4.13	**0.1**	x	✓	**4.3.1**
Alert Rate	0.92	0.19	1.51	0.22	0.59	**0.1**	x	✓	**4.3.3**
Pup-check Duration	3.40	0.88	4.24	1.02	0.84	**0.56**	x		**4.3.1**
Pup-check Rate	0.81	0.18	0.86	0.20	0.04	**0.89**	x		**4.3.3**
Random Focal	**n = 4**		**n = 5**						
Play	0.39	0.14	2.21	1.35	1.82	**0.43**	x		**4.3.2**
PINT	4.87	2.01	12.38	9.00	7.51	**0.69**	x		**4.3.2**
Alert Duration	11.09	3.56	6.38	2.57	4.71	**0.28**	x		**4.3.2**
Alert Rate	1.61	0.31	1.01	0.37	0.6	**0.28**	x		**4.3.3**
Pup-check Duration	4.55	0.63	3.67	1.09	0.88	**0.54**	x		**4.3.2**
Pup-check Rate	1.11	0.14	0.89	0.25	0.22	**0.49**	x		**4.3.3**
In-field Counts	**n = 10**		**n = 8**						
Pup-check rate /m^{-1}	0.80	0.13	0.74	0.14	0.05	**0.77**	x		**4.3.3**

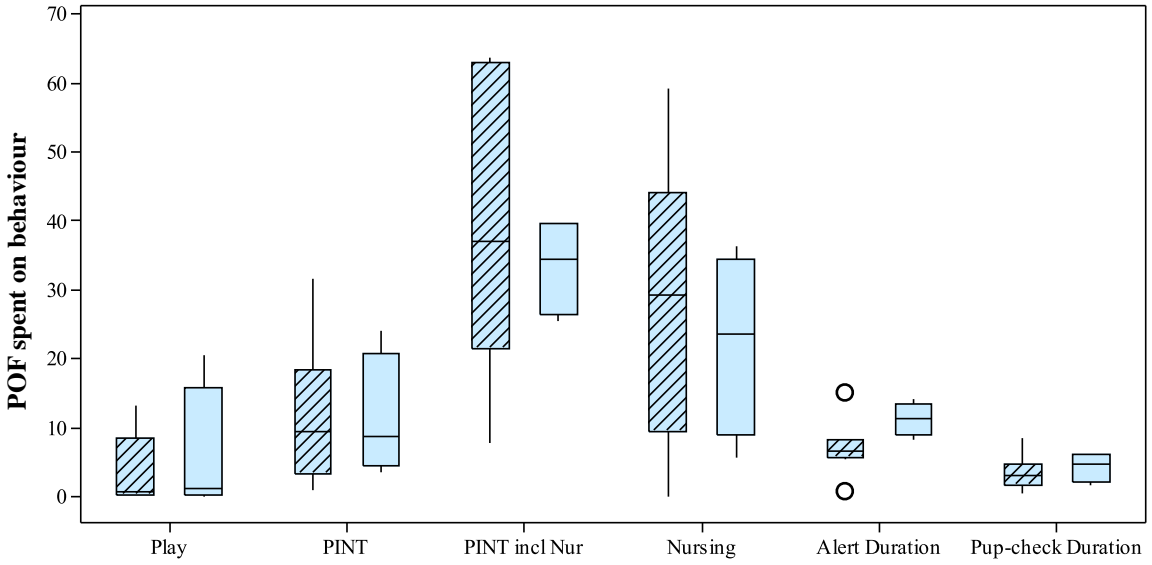


Figure 4.3.1 POF of nursing focal videos spent on broad category behaviours, compared between mothers of male pups (hashed bars) and those of female pups (clear bars). ○ represents an outlier.

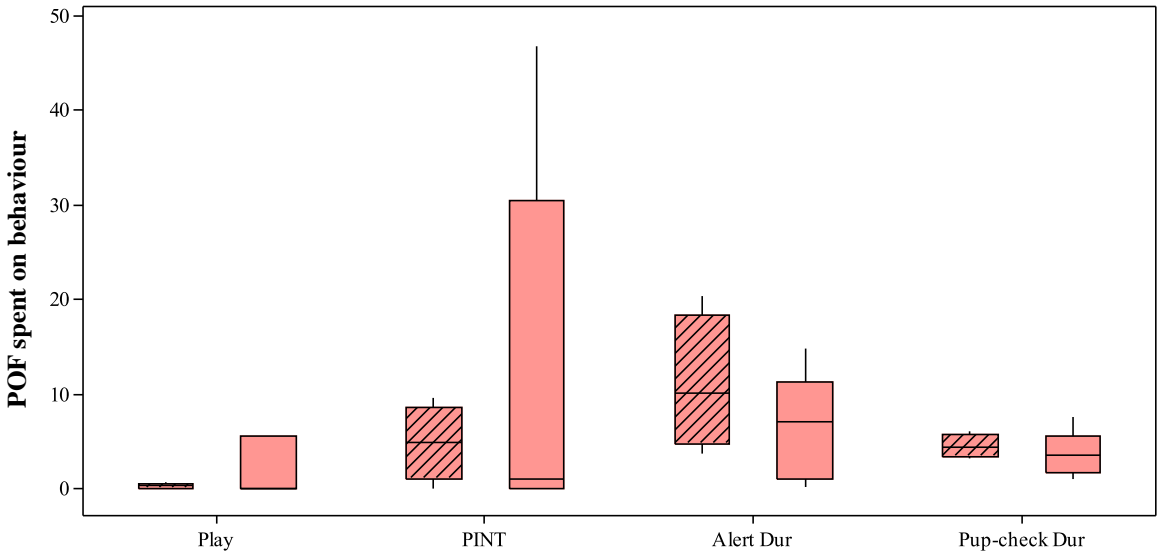


Figure 4.3.2 POF of random focal videos spent on broad category behaviours, compared between mothers of male pups (hashed bars) and those of female pups (clear bars).

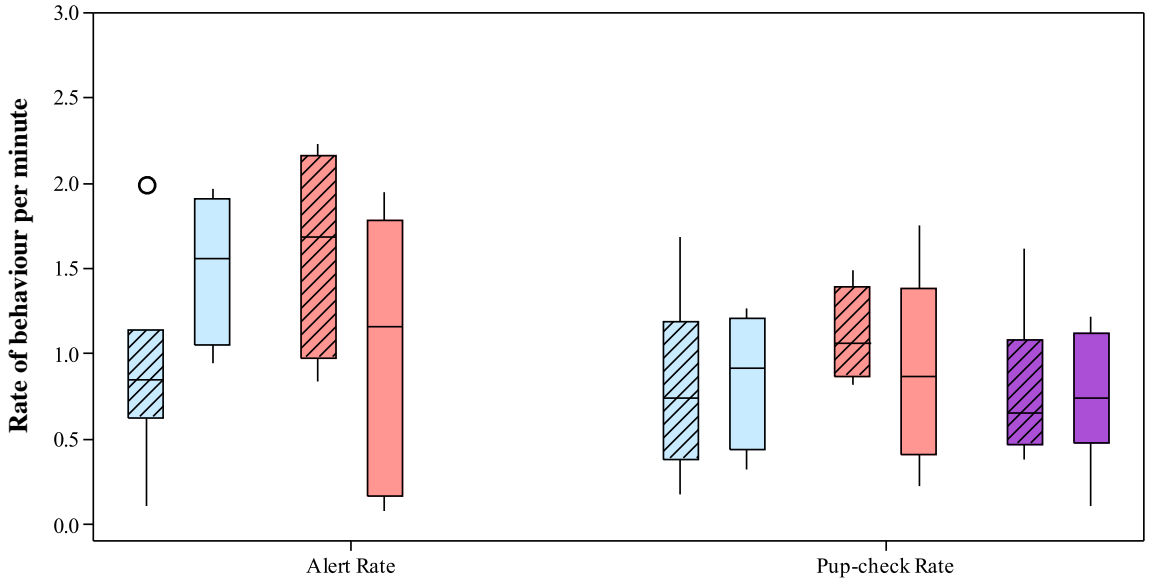


Figure 4.3.3 Rate per minute of alert and pup-check behaviours, compared between mothers of male pups (hashed bars) and those of female pups (clear bars). Rates taken during nursing (blue) and random (red) focal videos spent as well as pup-check rate per minute calculated from in-field counts (purple). ○ represents an outlier.

4.3.2 Environmental Disturbance

4.3.2.1 Regular Disturbance

The following analyses address question 3, set out in section 2.2.4: Is there an observable impact of regular human disturbance on maternal time budgets?

Females located on the public stretch of the colony were exposed for around twelve hours of each day to noise and visual disturbance caused by numerous human visitors to the beach. This was not experienced by mothers on the RAF beach. Although visitors to the public site were mostly kept behind a fence and away from the colony, occasionally photographers were allowed beyond this barrier, either moving within the main body of the colony or past it to the outer colony located on the tide line. Noise and movement from visitors remaining behind the barrier was constant from dawn up to and often exceeding dusk. This section of the analysis determines the presence, if any, of differences in maternal behaviours between mothers raising pups on the public site and those on the RAF site. **Table 4.3.2** shows the results of these comparisons.

In the comparison of nursing focal videos, there were no significant differences in maternal behaviours between mothers on either site. This is represented clearly by the box-plots in **Figure 4.3.4** and boxes shaded blue in **Figure 4.3.7**, in which the medians and interquartile ranges are fairly similar for public females and RAF females. The case is somewhat different when considering random focal behaviours. Comparisons for PINT, alert duration and alert and pup-check rates were carried out as before, comparing a combination of early and late lactation focal videos from public and RAF females. There was no significant impact of study site on these behaviours (see **Figure 4.3.6** and red boxes in **Figure 4.3.7**). However, during the initial comparison of early and late lactation behaviours within each site, significant differences in duration were observed between early and late lactation play (randomisation statistic = 4.12, $p = 0.035$) as well as early and late lactation pup-check duration (randomisation statistic = 3.04, $p = 0.02$), on the public site. For this reason, early and late lactation focal videos could not be combined, as they were for all other behaviours. Early measures of play and pup-check duration were compared between the public site and RAF site, followed by a comparison of late measures of the two behaviours across sites. Late pup-check duration was found to be significantly higher on the public site than on the RAF site (randomisation statistic = 2.91, $p = 0.04$). In addition, a trend for play behaviours to encompass a greater percentage of the focal during late lactation on the public site was observed, however this was not significant at the 0.05 level (randomisation statistic = 3.28, $p = 0.06$). These differences can be seen in **Figure 4.3.5**, the late lactation median values of play and pup-check duration behaviours differ greatly between the two sites.

Table 4.3.2 Outcomes of two-tailed randomisation tests (Todman & Dugard, 2001) comparing the percentage of time during focal videos spent performing behaviours or the rate of these behaviours per minute between the public and RAF sites. 'n' denotes sample size. In field pup-check rates were were compared using a Mann-Whitney U test.

Behaviour	**Public Site**		**RAF Site**		**Randomisation**			**Figure**
	Mean	S.E	Mean	S.E	Statistic	**p-value**	Significance	
							0.05 0.1	
Nursing Focal	**n = 10**		**n = 12**					
Play	4.44	2.17	1.86	0.67	2.59	**0.26**	x	**4.3.4**
PINT	9.90	3.10	10.95	2.39	1.04	**0.79**	x	**4.3.4**
PINT incl. nursing	30.51	5.94	27.80	3.79	2.71	**0.68**	x	**4.3.4**
Nursing	20.61	4.99	18.67	4.99	1.94	**0.8**	x	**4.3.4**
Alert Duration	6.61	1.60	9.94	1.36	3.32	**0.13**	x	**4.3.4**
Alert Rate	1.02	0.25	1.39	0.24	0.37	**0.34**	x	**4.3.7**
Pup-check Duration	3.59	0.73	3.47	0.56	0.12	**0.89**	x	**4.3.4**
Pup-check Rate	0.92	0.16	0.89	0.13	0.03	**0.89**	x	**4.3.7**
Random Focal	**n = 7**		**n = 10**					
PINT	6.42	2.76	12.26	5.39	5.83	**0.41**	x	**4.3.6**
Alert Duration	6.15	1.68	7.42	1.16	1.28	**0.51**	x	**4.3.6**
Alert Rate	0.95	0.21	1.13	0.17	0.18	**0.5**	x	**4.3.7**
Pup-check Rate	0.94	0.15	0.77	0.13	0.17	**0.38**	x	**4.3.7**

Table 4.3.2 cont. overleaf

Table 4.3.2 cont.

Behaviour	Public Site		RAF Site		Randomisation				Figure
	Mean	S.E	Mean	S.E	Statistic	p-value	Significance		
							0.05	0.1	
Early Random Focal	**n = 6**		**n = 7**						
Play Early	0.22	0.14	0.30	0.14	0.08	**0.73**	x		**4.3.6**
Pup-check Duration Early	2.74	0.41	2.61	0.70	0.13	**0.92**	x		**4.3.6**
Late Random Focal	**n = 4**		**n = 9**						
Play Late	4.33	2.43	1.06	0.68	3.28	**0.06**	x	✓	**4.3.6**
Pup-check Duration Late	5.78	1.25	2.86	0.52	2.91	**0.04**	✓		**4.3.6**

Behaviour	Public Site Median	RAF Site Median	Mann-Whitney U			Figure
			W value	p-value	Significance	
In-field Counts	**n = 24**	**n = 26**				
Pup-check rate / m^{-1}	0.53	0.52	633.5	**0.68**	x	**4.3.7**

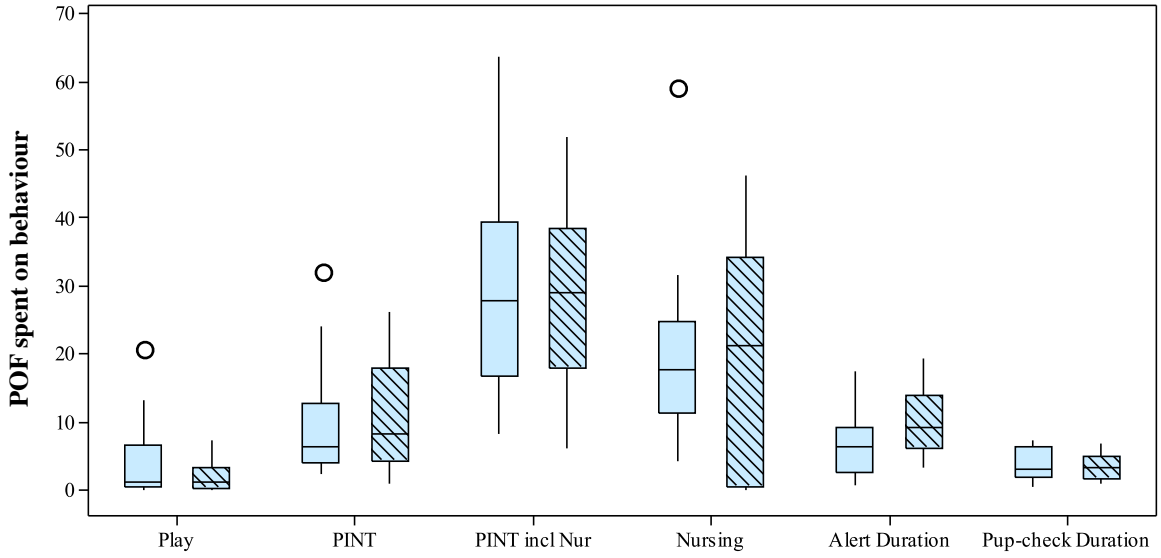


Figure 4.3.4 POF of nursing focal videos spent on broad category behaviours, compared between mothers on the public site (clear bars) and those on the RAF site (hashed bars). ○ represents an outlier.

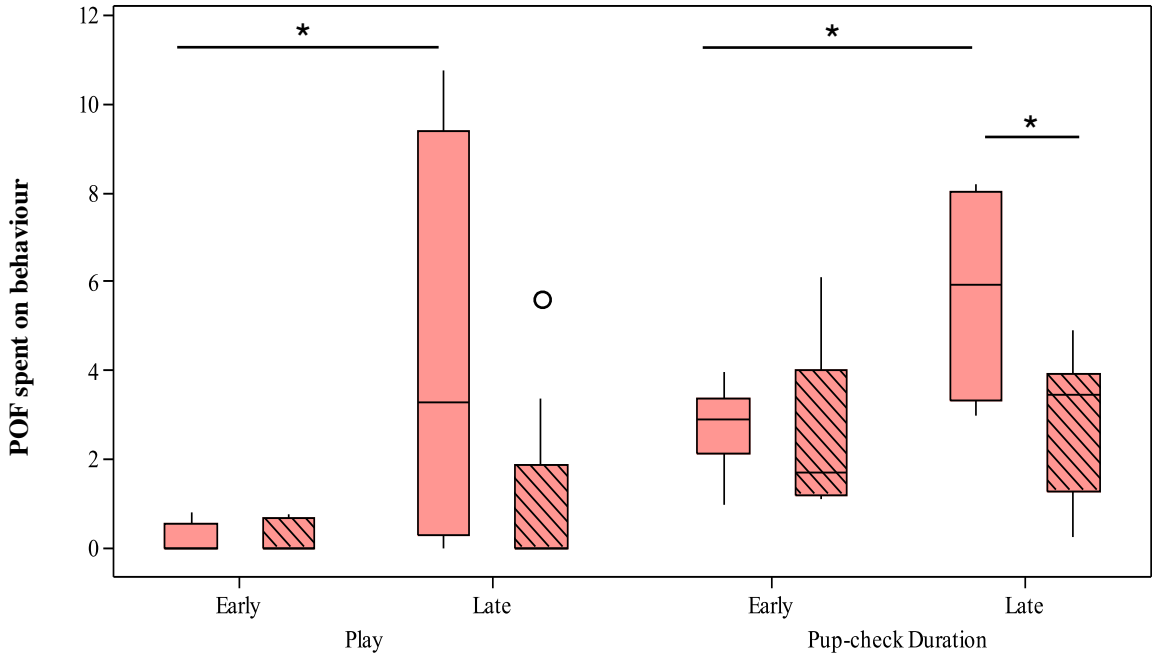


Figure 4.3.5 POF of random focal videos spent on play and pup-check behaviours, compared between mothers on the public site (clear bars) and those on the RAF site (hashed bars). These comparisons are separated into early and late lactation (shown by 'Early' and 'Late' on horizontal axis); significant differences were found between early and late measures for both behaviours on the public site, thus data could not be combined. ○ represents an outlier. * represents significance to the 0.05 level.

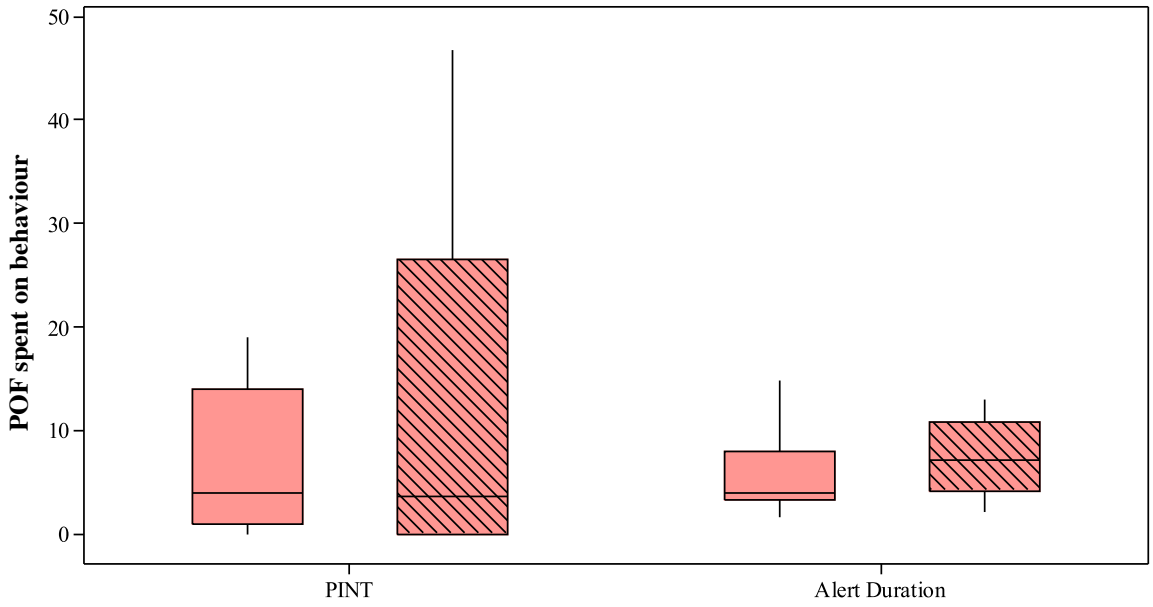


Figure 4.3.6 POF of random focal videos spent on PINT and alert behaviours, compared between mothers on the public site and those on the RAF site.

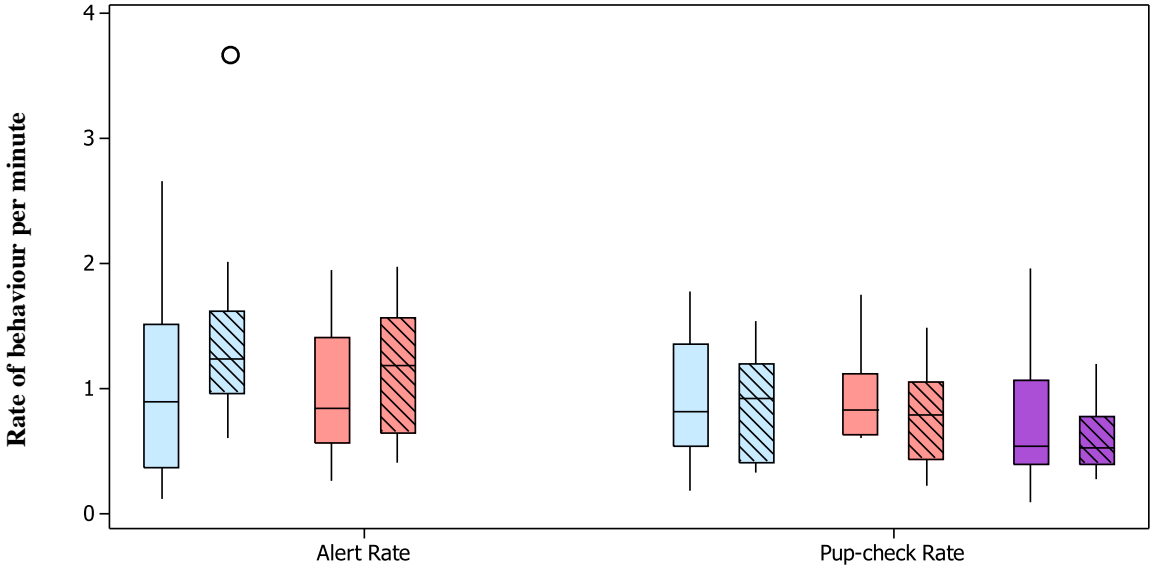


Figure 4.3.7 Rate per minute of alert and pup-check behaviours, compared between mothers on the public site and those on the RAF site. Rates taken during nursing (blue) and random (red) focal videos spent as well as pup-check rate per minute calculated from in-field counts (purple). ○ represents an outlier.

In-field pup-check counts were compared as rates per minute, between the public and RAF site. A Mann-Whitney U test was performed using Minitab 16. The results of this test did not suggest any significant effect of site on pup-checking behaviour ($W = 633.5$, $n_1 = 24$ $n_2 = 26$, $p = 0.68$).

4.3.2.2 Sudden Disturbance

The following analyses address all aspects of question 4, set out in section 2.2.4: Is there an observable impact of sudden environmental disturbance on maternal time budgets? If so, are these changes in behaviour mitigated or exacerbated (if changed at all) by prior exposure to the regular disturbance?

A new set of colour coding is used for box-plots throughout this final section (**Table 4.3.3**). An unexpected tidal storm surge on November 28th 2011 provided an opportunity to observe the impact of a sudden disturbance on the maternal behaviours of grey seals. Data collection took place over five weeks, the first three of which were before the storm surge (pre-tidal), while the final two fell after the storm surge (post-tidal). **Figure 4.3.8** gives a visual representation of the POF spent on each behaviour over the course of the five weeks. Todman & Dugard (2001) two-tailed randomisation test for small samples was used to compare POF spent on behaviours from before the storm surge against those after. Results of this analysis are shown in **Table 4.3.4**. Rates of alert and pup-check behaviours from focal videos were not included in this analysis. Because of the variation in duration of each occurrence of

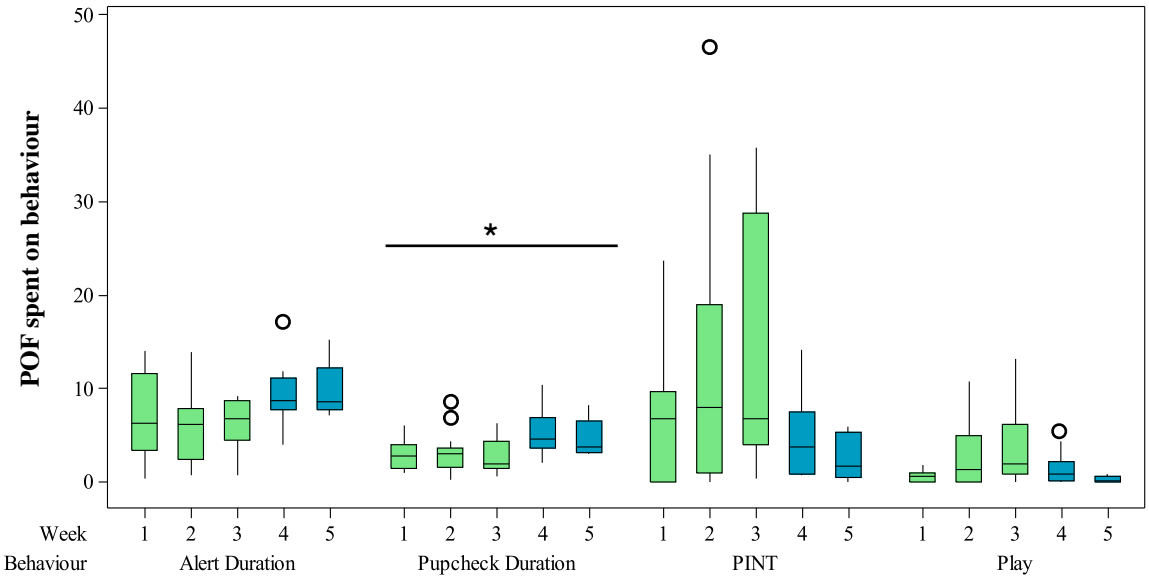


Figure 4.3.8 POF of nursing and random focal videos combined spent on broad category behaviours, compared across all 5 weeks of data collection. Weeks 1 - 3 (green) represent focal videos taken prior to the storm surge. Weeks 4 - 5 represent those taken after. ○ represents an outlier.

alert behaviour (**Table 4.2.1**), percentage duration provided a more reliable representation of overall levels of alert behaviours. Focal video pup-check rates were not included as in-field pup-check counts were deemed more accurate from previous analyses. Due to larger sample sizes, a Mann-Whitney U test was used to compare in-field pup-check rates. Comparisons between pre-tidal and post-tidal behaviours show a significant post-tidal increase in both measures of pup-check behaviours; pup-check duration from focal videos (randomisation statistic = 2.66, p = 0.002, **Figures 4.3.8** and **4.3.9**), and pup-check rates from in-field counts (W = 822.0, n_1 = 36 n_2 = 19, p = 0.001, **Figure 4.3.10**). Whilst POF spent alert after the storm surge was higher this was not significant (randomisation statistic = 2.96, p = 0.07). Median values were similar for POF spent on play between pre-tidal and post-tidal periods, as were median values for POF spent on PINT. The number of individuals within 5bl of the focal female was significantly higher pre-tidal than post (W = 3386.0, n_1 = 36, n_2 = 19, p = 0.009). **Figures 4.3.8** and **4.3.9** clearly show a far greater range in the POF spent on PINT prior to the storm surge, and a sudden reduction in the larger POF values for PINT after the storm surge. **Figure 4.3.8** shows a sudden drop in the higher values of POF spent on play between weeks 3 and 4. This is not represented in **Figure 4.3.9** or by the statistical analysis due to play values during week 1 reducing the overall average of pre-tidal play values. Responses to this sudden disturbance were compared between females on the public site and those on the RAF site to see whether exposure to regular disturbance influenced behavioural responses to sudden disturbance (**Table 4.3.5**). Behaviours were compared between sites during pre-tidal weeks and post-tidal weeks. Todman & Dugard's design 5a (2001) was used for all comparisons due to smaller sample sizes. Results suggest significantly more time spent alert on the RAF site prior to the storm surge. However, alert duration on the public and RAF sites become equal after the storm surge. **Figure 4.3.11** shows this increase in POF spent alert during post-tidal public focal videos. No other differences were found between public and RAF behaviours either prior to the storm surge or after it (shown by similar median values and data ranges in **Figures 4.3.11** and **4.3.12**). **Figure 4.3.13** shows the average alert duration (top) and average pup-check duration (bottom) calculated for each day. It also shows the average number of individuals within a 5bl radius of the focal female calculated per day. The increase in pup-check duration and trend toward an increase in alert duration can clearly be seen in **Figure 4.3.13** after the 28th of November.

Table 4.3.3 Colour coding for boxplots throughout section 4.3.2.2

Colour Key		Pattern Key	
Pre-Tidal		Public Site	
Post-Tidal		RAF Site	

Table 4.3.4 Outcomes of two-tailed randomisation tests (Todman & Dugard, 2001) comparing the percentage of time during focal videos spent performing behaviours / the rate of these behaviours per minute between pre- and post-tidal periods. 'n' denotes sample size. In field pup-check rates were compared using a Mann-Whitney U test as sample size were larger. The results are given at the bottom of this table in a separate section.

Behaviour	**Pre-Tidal**		**Post-Tidal**		**Randomisation**				**Figure**
	Mean	S.E	Mean	S.E	Statistic	**p-value**	Significance		
							0.05	0.1	
Focal Videos	**n = 22**		**n = 10**						
Play	1.77	0.64	1.47	0.59	0.31	**0.81**	x		**4.3.9**
PINT	10.36	2.26	4.94	1.34	5.42	**0.14**	x		**4.3.9**
Alert Duration	6.61	0.91	9.57	1.12	2.96	**0.07**	x	✓	**4.3.9**
Pup-check Duration	2.54	0.30	5.20	0.76	2.66	**0.002**	✓		**4.3.9**

Behaviour	**Pre-Tidal Median**	**Post-Tidal Median**	**Mann-Whitney U**			**Figure**
			W value	**p-value**	Significance	
In-field Counts	**n = 36**	**n = 19**				
Pup-check rate /m^{-1}	0.41	0.8	822.0	**0.001**	✓	**4.3.10**
Number of individuals within 5bl	9.00	7.00	3386.0	**0.009**	✓	**4.3.10**

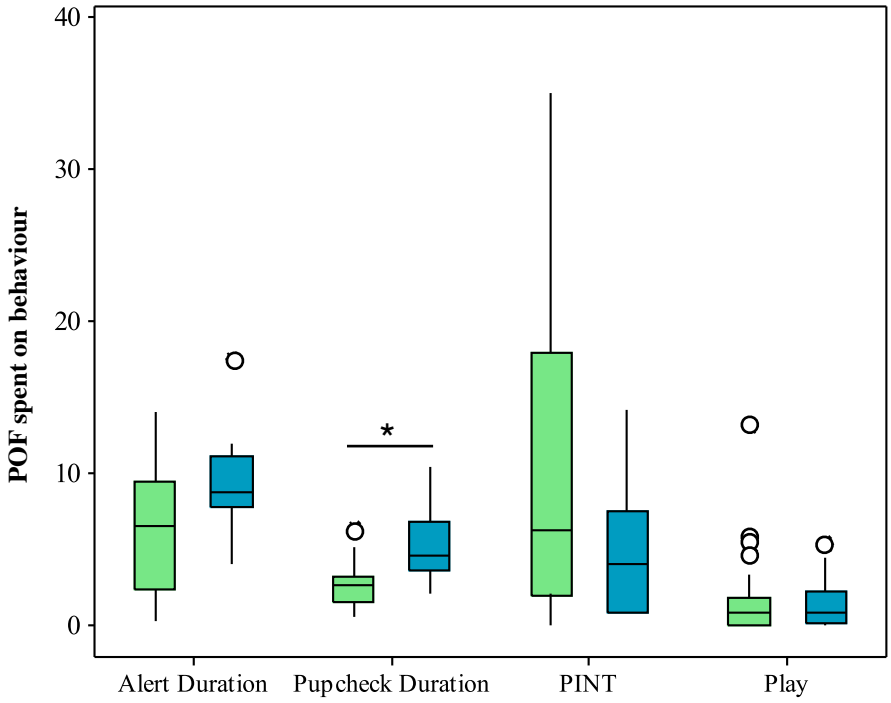


Figure 4.3.9 POF of nursing and random focal videos combined spent on broad category behaviours, compared between pre-tidal (green) and post-tidal (blue) weeks. O represents an outlier. * represents significance to the 0.05 level.

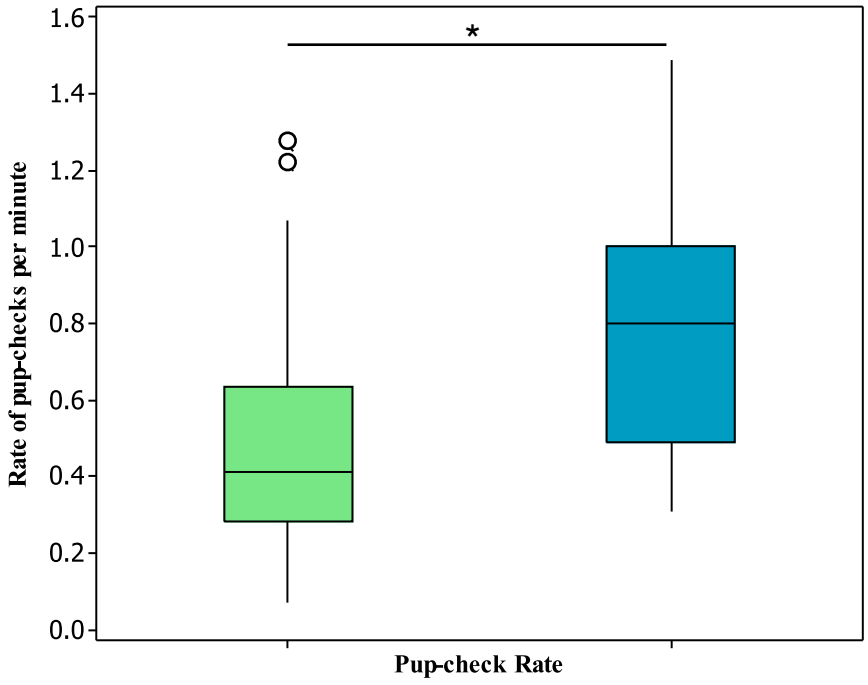


Figure 4.3.10 Rate per minute of in-field pup-checks, compared between pre-tidal (green) and post-tidal (blue) weeks. O represents an outlier. * represents significance to the 0.05 level.

Table 4.3.5 Outcomes of two-tailed randomisation tests (Todman & Dugard, 2001) comparing the percentage of each focal spent performing behaviours / the rate of these behaviours per minute between the public and RAF sites. Comparisons are separated between pre- and post-tidal data. 'n' denotes sample size. In field pup-check rates were compared using a Mann-Whitney U test as sample size were larger. The results are given at the bottom of this table in a separate section.

Behaviour	**Public Site**		**RAF Site**		**Randomisation**			**Figure**
	Mean	S.E	Mean	S.E	Statistic	**p-value**	Significance	
							0.05 0.1	
Pre-tidal Focal	**n = 10**		**n = 12**					
Play	2.41	1.31	1.24	0.45	1.17	**0.43**	x	**4.3.11**
PINT	9.20	3.20	11.33	3.27	2.12	**0.65**	x	**4.3.11**
Alert Duration	4.18	1.17	8.64	1.05	4.46	**0.02**	✓	**4.3.11**
Pup-check Duration	2.41	0.44	2.65	0.42	0.24	**0.68**	x	**4.3.11**
Post-tidal Focal	**n = 6**		**n = 4**					
Play	1.92	0.96	0.79	0.30	1.13	**0.43**	x	**4.3.11**
PINT	5.44	2.13	4.20	1.35	1.24	**0.67**	x	**4.3.11**
Alert Duration	10.70	1.44	7.88	1.63	2.82	**0.29**	x	**4.3.11**
Pup-check Duration	6.08	1.07	3.88	0.71	2.20	**0.18**	x	**4.3.11**
Pre-tidal In-field Counts	**n = 20**		**n = 16**					
Pup-check Rate /m^{-1}	0.50	0.08	0.44	0.04	0.06	**0.54**	x	**4.3.12**
Post-tidal In-field Counts	**n = 8**		**n = 11**					
Pup-check Rate /m^{-1}	0.85	0.14	0.75	0.09	0.11	**0.53**	x	**4.3.12**

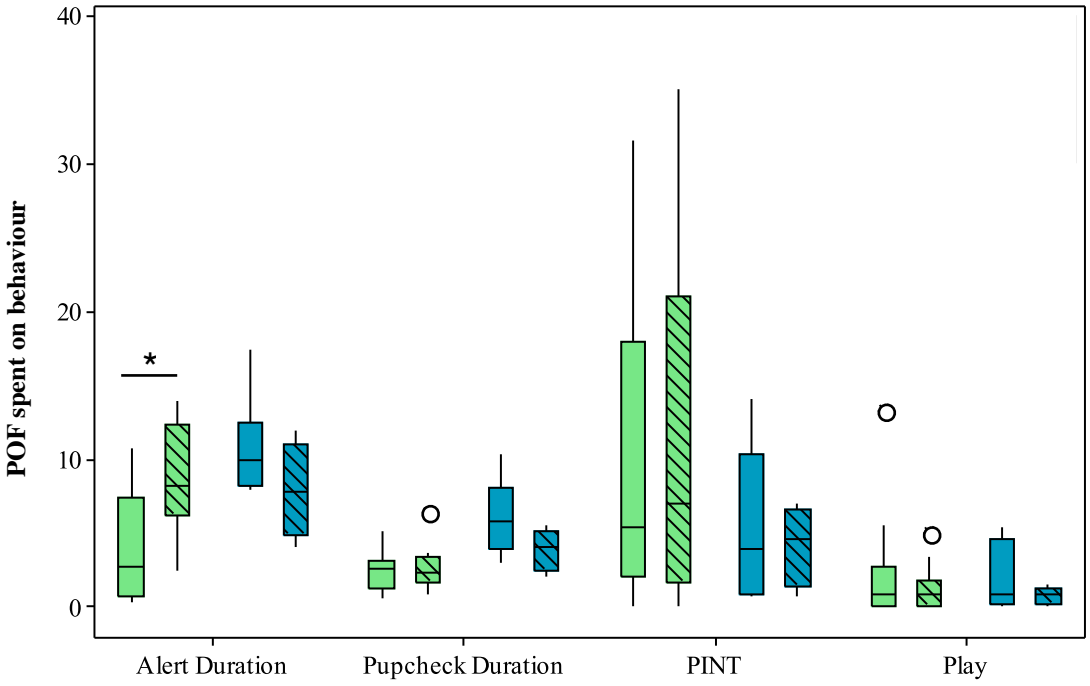


Figure 4.3.11 POF of nursing and random focal videos combined spent on broad category behaviours, compared between the public (clear bars) and RAF (hashed bars) sites, separated into pre-tidal (green) and post-tidal (blue) weeks. O represents an outlier. * represents significance to the 0.05 level.

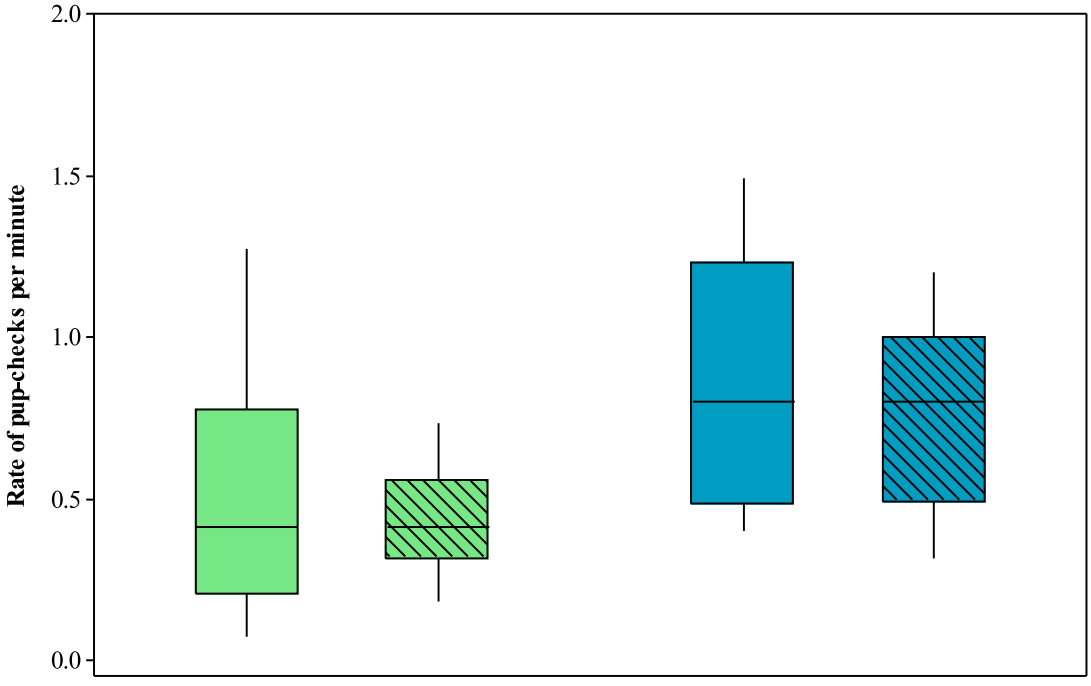


Figure 4.3.12 Rate per minute of in-field pup-checks, compared between the public (clear bars) and RAF (hashed bars) sites, separated into pre-tidal (green) and post-tidal (blue) weeks. O represents an outlier.

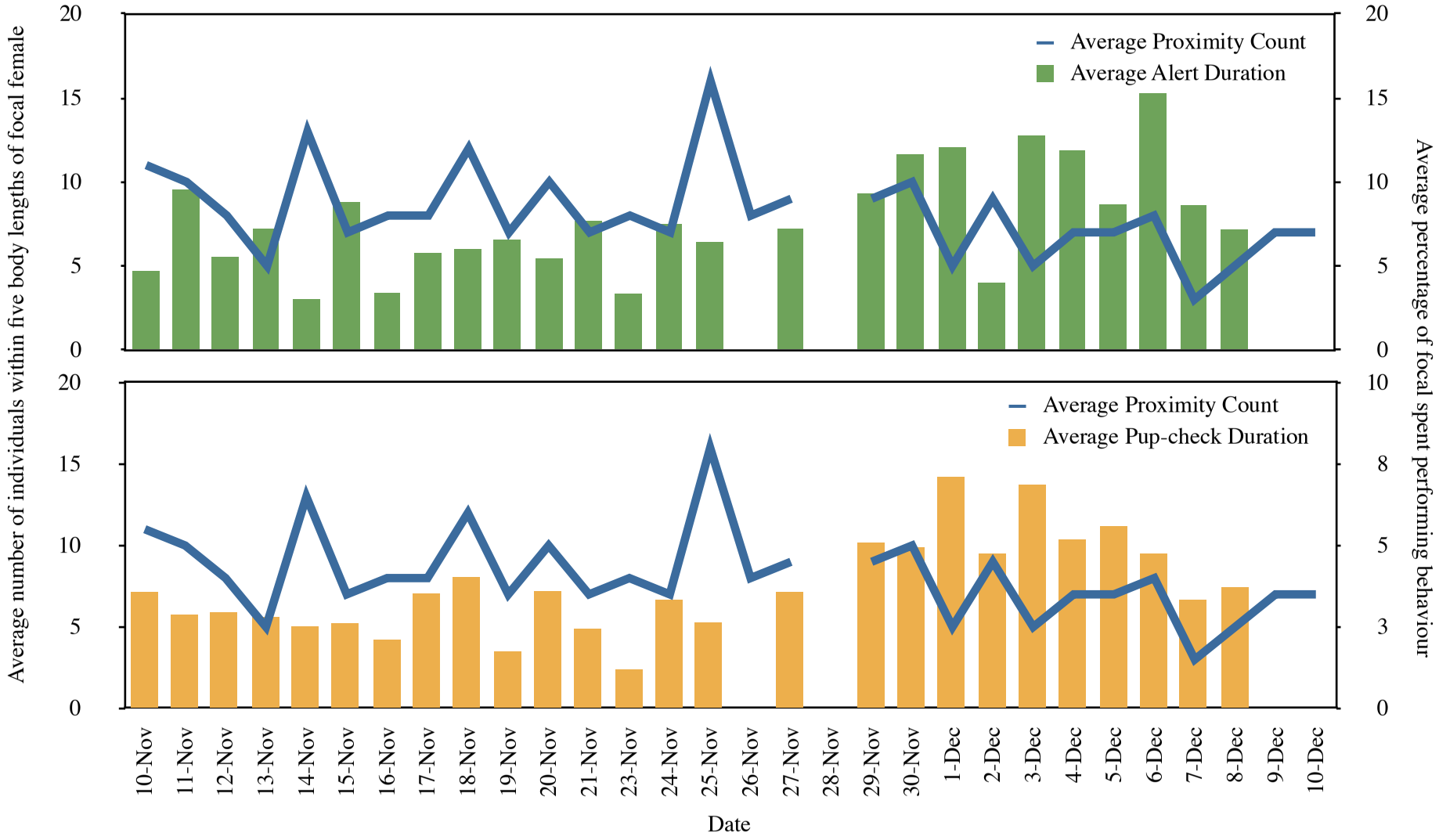


Figure 4.3.13 Daily averages for vigilance behaviours and counts of the number of individuals within a 5bl radius of the focal female. Upper chart shows alert duration (green) and lower chart pup-check duration (yellow). The storm surge is represented by a gap in the data on the 28th November. (Lack of duration data on the 26th, 9th and 10th due to unsuccessful videos from these days).

5. DISCUSSION AND INTERPRETATION

Overall, analyses provide preliminary evidence of mothering styles within grey seal breeding colonies. Results identify consistent individual differences (CIDs) in mother-pup interactions (PINT), both including and excluding nursing, as well as nursing alone. These CIDs are apparent during periods of directed mother-pup interaction (represented by nursing focal videos), but not at other times (i.e. random focal videos). Play behaviours were not found to be repeatable, suggesting that play may be either common to all seal mothers or situation dependent. However, given the small values for average POF spent on play, and the infrequent occurrence of play behaviours (personal observation), 30 minute focal videos may have been unsuitable for identifying CIDs in play behaviours. This unsuitability is further discussed in section 5.3.1.1. CIDs were identified in pup-checking behaviours from in-field counts, in agreement with previous literature (Twiss *et al*., 2011; Twiss *et al*., 2012). Pup-check counts and rates from focal videos did not support this finding, though potential reasons for this disagreement in data are plentiful and will be discussed during this chapter.

Results provide no evidence to support differential maternal behavioural investment according to pup gender. The contrasting levels of human disturbance appears to have little effect on maternal behaviour when the field season is considered as a whole. There is some evidence for a higher percentage of focal (POF) spent pup-checking and a trend towards more play behaviour on the public site during late lactation.

There was a significant rise in pup-checking behaviours, both duration and rate, after the storm surge, and a trend toward a greater duration of alert behaviours. Results suggest that females located on the RAF stretch of the colony spent more time alert prior to the storm surge than did those on the public site.

Figure 5.0.1 Mother and pup on the RAF site about to begin a play session. Pup is displaying the grey seal species-characteristic invitation to play.

However, after the storm surge, alert duration increased on the public site and was equal between the two sites. This suggests a degree of flexibility with regards to this behaviour.

5.1 IDENTIFYING MOTHERING STYLES

5.1.1 Differences in maternal time budgets across lactation stages

The current study suggests that mothers spend more time interacting with and nursing older pups (stage 3 and 4) compared with younger pups (stage 1 and 2). Nursing alone appears to increase in duration as pups grow older. This is contrary to previous studies in Orkney which reported no difference in nursing duration throughout lactation (Fogden, 1971).

It is tempting to discuss the increase in terms of greater maternal expenditure in older pups, and in some ways this would appear intuitive. An older and therefore larger pup should have a greater capacity for the intake of milk, thus would be expected to spend longer nursing. However, this study did not take any measure of milk content or transfer efficiency, and so care must be taken when discussing greater nursing duration in terms of an increase in maternal expenditure. It is possible that milk transfer efficiency reduces with reduced maternal resources (toward the end of the fasting period), and that longer nursing periods are employed to account for this. As mothers fast, the content of their milk sees an increased proportion of fat and energy and a reduction in the proportion of protein (Iverson *et al*., 1993; Mellish *et al*., 1999). It may be that the increased POF spent nursing seen in late lactation mother-pup pairs is a mechanism to ensure that protein transfer to the pup is still sufficient. Certainly we can describe the increased nursing POF as an increase in maternal time investment. For these reasons nursing, as defined by the current study, may be better considered as another mother-pup interaction and not a behavioural category in its own right.

The increase in overall mother-pup interaction (including nursing) from early lactation to late lactation is likely a result of the greater co-ordination and mobility of older pups, making them better able to respond to and/or initiate maternal interactions.

Play behaviours alone do not appear to change in duration between early and late lactation. Grey seal mothers generally suckle only their own pup (section 2.1.1). For this to occur, a bond must be formed from mother to pup, in order to recognise the pup prior to nursing. Smell and, to a lesser extent, pup vocalisations are used by the mother to form this bond (Fogden, 1971; Caudron *et al*., 198; Insley *et al*., 2003). Play behaviours, including the species characteristic invitation to play, have been suggested as a form of bonding between juvenile grey seals (Wilson, 1974); these behaviours maintain contact between the muzzle of one individual and the throat, chest or back-of-the-head of the other, regions which emit higher levels of scent (Wilson, 1974). Mothers (and pups of later stages; **Figure 5.0.1**) were seen performing

this species characteristic invitation to play, associated with rolling, nosing and play-snapping sessions. It is possible that mothers too use play to maintain the bond between themselves and their pup, and that this is why play is performed equally throughout early and late lactation. Other mammals with parental care lick and groom their offspring to stimulate various physiological developments within the offspring. Champagne *et al*. (2007) report the use of licking and grooming by mice to stimulate movement of the young, cause alterations to the body temperature as well as to encourage defecation and suckling. Neither licking nor grooming behaviours are performed by grey seal mothers. Flippering has been suggested as a mechanism by which grey seal mothers encourage the pup to suckle (Fogden, 1971; Kastelein *et al*., 1991). In-field observations during the current study suggested that, when flippering was unsuccessful, the mother may rest her head on the pup in the invitation to play stance, begin nosing, play-snapping and eventually rolling with the pup. This would then be followed by another bout of flippering. Often the pup would nurse after the second bout of flippering (personal observation). Nosing, play-snapping and rolling caused the pup to become physically active (see **Figure 5.1.1***a., c.* & *d.*), moving its head or escaping from under the mother's body, and therefore has the potential to alter its body temperature. It is possible, then, that

Figure 5.1.1 Building a bond and assisting pup development? Photographs of *a*. Nosing; *b*. Smelling; *c*. Playing on land; *d*. Playing in water. Photographs shows the proximity of the mother's muzzle to the pup's upper body throughout the duration of these behaviours, potentially using scent to maintain the bond between mother and pup. Pictures *c*. and *d*. also show the effect of the 'rolling' behaviour on pup movement.

the effects of flippering and play behaviours in grey seals are homologous to grooming and licking in other mammals in terms of offspring development.

There are difficulties putting findings of the current study into the context of existing literature. Behavioural time budgets for male grey seals are readily available (for example Boness, 1984; Tinker *et al*., 1995; Twiss & Franklin, 2010). The same cannot be said of maternal behaviours for the females of the species. Fogden (1971) produces an in-depth description with visual support for smelling, flippering, presenting and nursing behaviours. Kovacs (1987) provides time budgets for various maternal behaviours on the Isle of May, accounting for nutritional and vigilance behaviours, as well as solitary behaviours and resting periods. Results from Kovacs' study are split between three different sites and according to sex of the pup making overall comparison with the current study problematic.

Qualitative comparisons can be made between Kovacs' 1987 study and this thesis. Mothers at Donna Nook spent, on average, longer alert than those in Kovacs' study (7% of total time compared to 5% on the Isle of May). During the current study, percentage of time spent nursing was also higher than that found by Kovacs. Nursing duration remained between 8 and 12% of overall time on the Isle of May, whilst on Donna Nook the average nursing duration was 14% during early lactation, increasing to 24% during late lactation. This significant increase in nursing duration was not observed by Kovacs. Other mother-pup interactions have been omitted from Kovacs' (1987) report, with no mention of playing or negative interactions between the pair.

Twiss *et al*. (2000) used maternal behavioural categories, similar to those in the current study, when assessing behaviour on the Isle of May. Two sites were assessed on the Isle of May: West Rona Beach and Tarbet. Categories used were: resting, alert, aggression (non-sexual), interacting with pup (PINT including nursing in current study), locomotion or involved in sexual interactions. Data were collected using scan samples at 2 minute intervals over a number of 30 minute periods. Percentage of scans recording each behavioural category was calculated for all 30 minute samples. These data are not directly comparable to the results obtained from the current study, as the 30 minute sample periods neither specifically include nor exclude nursing bouts as in the current study. Twiss *et al*., (2000) measure the percentage of scans within 30 minutes as opposed to the percentage of the total time. Nevertheless, comparisons can be drawn to the current study. By selecting a sample of nursing and random focal videos (combining data from periods of directed mother-pup interaction with periods of non-directed interaction as was used by Twiss *et al*., 2000), data can be compared qualitatively to that collected on the Isle of May.

Table 5.1.1 Comparison of PINT (including nursing) between mothers on the Isle of May (Twiss *et al*., 2000) and on Donna Nook (current study)

Measure	Percentage of scan samples during a 30 minute period identified as PINT (Twiss *et al*., 2000)		POF spent on PINT
	West Rona Beach	Tarbet	Donna Nook
Median	5.19	4.55	18.53
Lower Range Limit	0	0	0
Upper Range Limit	31.82	35.48	62.53

Table 5.1.1 displays data medians and ranges for females on both sites on the Isle of May and females at Donna Nook.

This comparison suggests far higher levels of mother-pup interaction at Donna Nook compared to the Isle of May. This statement must be made with care; the nature of data collection on the Isle of May was discrete, such that shorter mother-pup interactions (for example, play behaviours) may not have been identified by the 2-minute interval scans. Data collection during the current study was continuous and so accounted for all mother-pup behaviours within the 30 minute focal.

However, the results may still be indicative of different maternal behaviour according to breeding site. The Isle of May has a rocky topography, with water available only in isolated pools, similar to that of North Rona (Redman *et al*., 2001). Studies of grey seal mothers on North Rona describe individuals travelling great distances and spending time separated from their pup in order to gain access to water (Redman *et al*., 2001). The same is true on the Isle of May, and those mothers with limited access to the sea spend far less time in attendance with their pup (Twiss *et al*., 2000). At Donna Nook, the tide line remains around a mile from the inner colony. However, the sediment on which the colony forms can be easily manipulated by digging to expose a cool under-layer. This, in conjunction with occasional nearby water collection in dips and trenches, may allow mothers at Donna Nook access to *in situ* thermoregulation. It is also possible that there is a differential need for thermoregulation between Donna Nook and the Isle of May due to weather conditions, however the breeding season on both sites is similar so it is unlikely that temperature would differ extensively between them.

During the current study, proximity maps identified only three occasions during which a mother was found outside of a 5bl radius of her pup. Mothers at Donna Nook were rarely seen moving long distances from their pup,

and as such the opportunities for mother pup interactions were greatly increased.

5.1.2 Consistent individual differences in maternal behaviour

Consistent individual differences (CIDs) were measured from early to late lactation, with the intraclass correlation coefficient (ICC) used as the measure of repeatability (Bell *et al*., 2009). Mothers at Donna Nook showed CIDs in the amount of time spent interacting with their pups. This includes both positive and negative interactions and becomes particularly obvious when time spent nursing is included. These CIDs were only present during times of directed mother-pup interactions (nursing focal videos) but not at other times (random focal videos).

Similarly, mother-offspring relationship quality (MORQ) index values calculated from nursing focal videos were found to be consistent between early and late lactation. This result suggests that those mothers that show their pups greater affiliation than rejection (relative to other mothers on the colony), do so consistently from early lactation to late lactation. The same can be said for those mothers showing their pup more rejective behaviour than affiliative. Four mothers, (P7, P20, R21 and LINDA) were identified as being more affiliative than others within the study, and as such conclusions could be drawn that these four mothers were the most experienced and successful. This conclusion would be making the assumption that affiliative mother-pup interactions always positively influence the development of the pup. To do this, the impact of maternal behaviour on pup behaviour would first need to be assessed, and the desirability of the pup behaviour, in terms of future survival, qualified. As discussed in the introductory chapter, section 1.4.2, guinea pig pups which experienced extended periods of time without maternal input were found to have greater boldness than those which had enjoyed extensive maternal contact prior to weaning (Albers *et al*., 2000*b*). Similarly, boldness and enterprise were found to be positively linked to maternal rejection in both *M. fuscata* and *M. mulatta* (Bardi & Huffman, 2006). The latter paper suggested that maternal behaviour should become progressively less responsive toward the infant in order to encourage offspring independence (Bardi & Huffman, 2006). High maternal affiliation in grey seals, therefore, cannot be judged to be a result of experience and previous rearing success, as corresponding data on the influence on pup development is not available. Instead, by considering pupping dates, we can speculate on the age and experience of these four females. The body mass of female grey seals increases with age (Bowen *et al*., 2006). Anderson & Fedak (1987) and Pomeroy *et al*. (1999) both identified a tendency for larger females to pup earlier in the season. With this information it may be postulated that older mothers pup earlier in the season than

younger ones. For mothers in this study, there seems to be no correlation between relative pupping date (estimated from date at which pup was recorded as stage 2) and MORQ values. P7, P20, R21 and LINDA's pupping dates were spread throughout November, suggesting that a high MORQ value was not the result of a particularly early or late birth. Mothers with more affiliative relationships did not check their pup significantly more or less than those with more rejective relationships, nor did they frequently retain their pup at a closer proximity. Thus affiliative mothers are not necessarily more protective of their offspring. This may reduce the potential negative impacts of a more interactive mother-offspring relationship. In order to assess whether the effects of these affiliative behaviours on the offspring are positive or negative, data would need to be collected from the pup itself. Suggestions for this are made in the extensions section (5.3.2).

CIDs were identified in time spent nursing across early and late lactation. As mentioned previously, no measure of milk transfer efficiency or milk content were collected during this study and so it cannot be concluded that certain mothers expended more resources through nursing than others. It is possible that mothers spending repeatedly more time nursing were, in fact, less efficient at transferring milk to their pup. Mellish *et al*. (1999) identified variation between mothers in milk content with respect to fat and protein. Consistent differences in nursing times, identified in the current study, could be attributed to this variation in milk content, requiring pups to suckle for different periods to ensure that they obtain the correct balance of nutrients.

Alert and Pup-check duration were not found to be repeatable. In the case of alert duration, this is likely due to the high intra-individual variation in duration of each bout of behaviour. Mothers were highly variable in the length of time each bout of alertness lasted (represented by the large coefficients of variance, **Table 4.2.1**). Neither pup-check nor alert rates were found to be repeatable; though during nursing focal videos, alert rates showed a tendency towards repeatability and similarly for pup-check rates during random focal videos, neither of these were found to be significant. It is possible that these behaviours are entirely responsive to environment and fluctuate depending on the situation. However, CIDs have previously been identified in pup-checking behaviour (Twiss *et al*., 2011; Twiss *et al*., 2012) and in calculations using in-field counts of pup-checks during the current study.

There are a number of possibilities for the lack of repeatability observed in pup-check rates from focal videos. Twiss *et al,* (2012) compared consistency in pup checking behaviour across situations (as defined by Sih *et al*., 2004; given in **Table 1.1.1**) in the North Rona colony. During 2009 and 2010,

individually identifiable females were exposed to a novel aural stimuli; a remote control vehicle (RCV) playing a 'wolf' call. Pup-check rate was extracted using a video focal lasting eleven minutes (Twiss *et al*., 2011). Relaxed pup-check rate was collected from these same known females in 2010 using thirty minute video recordings, only taken when the mother was undisturbed by other members of the colony. Pup-check behaviour in response to the stimulus (disturbed situation) was found to be consistent within individuals from year to year, so too was pup-check behaviour in relaxed situations. However, no relationship could be found between pup-check behaviour in a disturbed situation when compared to a relaxed situation (Twiss *et al*., 2012). This has been linked to the mothers' personal behavioural types being either proactive or reactive. Proactive mothers respond less to environmental change and perform pup-checks at a fairly constant rate irrespective of situation; reactive mothers respond to the presence of the disturbance stimuli and alter their pup-check rate accordingly (Twiss *et al*., 2011; Twiss *et al*., 2012).

Video recordings taken during the current study were collected according to the behaviour of the focal mother and her interactions with her pup, the behaviour of the surrounding colony was noted but did not dictate the time at which the videos were taken. Disturbance was not quantified during the current study, but likely varied between focal videos. For example, we can consider the focal female becoming involved an aggressive interaction with another adult on the colony as a form of disturbance. If this is the case, just over half of focal females had early and late nursing focal videos taken in the same situation (either both or neither including disturbance). Providing the colony at Donna Nook is similar to that on North Rona, with female behavioural types spanning across the proactive-reactive axis, the presence of any disturbance or aggression during the focal would affect pup-checking rates differentially, depending on whether the female had a proactive or reactive behavioural type. This reasoning becomes more interesting when we consider that 80% of females have early and late lactation random focal videos from the same situation, and that pup-check rates in this case show a trend towards repeatability ($p = 0.07$).

This reasoning does not explain the positive identification of CIDs from the in-field pup-check counts and rates during the current study. In-field pup counts were made at the same time each day, again regardless of colony aggression or disturbance. If the above explanation for the lack of CIDs in focal pup-check rates were true, it would be expected that in-field pup-check rates would suffer from the same limitations. This is clearly not the case, as both in-field pup-check rates and pup-check counts in 15 minutes were significantly repeatable. To provide an explanation for this, the methods

of data collection must be considered in some detail.

Firstly, three in-field pup-check counts were made per day. This in itself would increase the power of the test. When calculating CIDs in the number of pup-checks made in 15 minutes, each count was included individually into the ICC, making a total of 6 data points per female compared to the 2 used with focal videos. However, in-field pup-check rates combined the three daily pup-check counts and were calculated from the total. In-field pup-check rates therefore had only two data points entered into the ICC. Though the significance decreased for in-field rates compared to counts, there was still a positive identification of CIDs in this behavioural measure. For this reason we must conclude that the number of data points is not entirely accountable for the difference between in-field and focal pup-check rates.

Secondly, each round of pup-check counts lasted 15 minutes (making a total of 45 minutes each day) but were separated by an hour each. As a result, these spanned a greater proportion of the day than a single thirty minute focal. Each daily set of pup-check counts is therefore likely to encompass both an element of relaxed and of disturbed pup-checking. The more even spread of data collection throughout the day may ensure that the data collected between early lactation and late lactation is more comparable in terms of situation (i.e. in-field counts should be less influenced by stochastic events or disturbances, unlike a single 30 minute focal).

Finally, we must accept that there may be some lack of clarity in counts made from the video recordings. Pup-checks are quick and sometimes subtle movements. To identify a pup-check, the exact location of the pup must be known. Limited field of vision and poorer sense of depth provided by the recordings makes this difficult. Pup-checks are far easier to determine using in-field counts; this was the initial rationale behind taking these.

The presence of CIDs in mother-pup interactions throughout a single breeding season is preliminary evidence for the existence of mothering styles in grey seals. This lends weight to previous studies, supporting evidence of CIDs in maternal behaviour. This evidence is strengthened by CIDs found during in-field pup-checking, as well as in pup-check counts from previous literature (Twiss *et al*., 2011; Twiss *et al*., 2012). The consistency in MORQ index values, calculated from nursing focal videos, adds further support to the existence of mothering styles. The current study lends weight to previous studies, supporting evidence of CIDs in the maternal behaviours of grey seals. Further data would be required to confirm the existence of CIDs mothering styles. Possible extensions to this study are discussed in section 5.3.2.

5.2 POSSIBLE DRIVERS OF MATERNAL BEHAVIOUR

5.2.1 Pup Gender

This study identified no differences in maternal attendance behaviours between mothers rearing male pups and those rearing female pups. This contradicts findings of a number of studies around Britain, but supports many of those based outside of the UK (Smiseth & Lorentsen, 1995*a*). It is generally expected that mothers with male pups should show higher levels of maternal investment than those with female pups. This is because larger adult males tend to have a higher fitness than smaller males, but body size has less effect on the overall reproductive success of a female. There have, however, been a number of suggestions for why this difference in maternal investment may not occur. Anderson & Fedak (1987) carried out behavioural observations as well as weighing grey seal mothers and pups on the island of North Rona. Though their results suggested greater weight gain by male pups than female pups, and greater weight loss for mothers of these male pups than those of the female pups, their behavioural observations suggested no significant differences in nursing rates between male and female pups. If nursing duration is not different, it is possible that another activity, perhaps a mother-pup interaction which would maintain the correlation between weight loss and weight gain, is responsible for this difference between mothers of male pups and those of female pups. However, this difference in mother-offspring behaviour between pup genders is not supported by results from the current study; results show that within the focal group at Donna Nook, no social maternal behaviours were altered by the gender of the pup.

Previous authors have suggested that across a number of phocid seals the levels of maternal attendance, especially nursing, are partially dependent on the behaviour of the pup (Arnbom *et al*., 1997; Smiseth & Lorentsen, 2001). A study on begging calls made by grey seal pups adds some support to this. Smiseth & Lorentsen (2001) observed that mothers tended to show increased locomotion toward the pup after a begging call had been made than at any other time. However, suckling rate was not correlated to the number of begging calls made, suggesting that maternal attention is gained by producing the begging call, but nursing does not always follow this (Smiseth & Lorentsen, 2001). In an earlier paper, Smiseth & Lorentsen (1995*a*) observe a lack of effect of pup gender on grey seal maternal behaviour while studying a colony on Froan, Norway, with the exception that mothers with male pups spent more time in 'visual contact' with their pups. Interestingly, the results from the current study suggest a slight trend towards mothers with female pups spending, on average, a larger proportion of their time performing alert behaviours. The

rate of these alert behaviours per minute is slightly higher for mothers with female pups. However, neither of these results is statistically significant. Smiseth & Lorentsen (1995*a*) suggest that maternal investment did not bias one pup gender over another because weaning weight has little input into the final adult weight, a factor which has previously been considered in studies on southern elephant seals. Instead, weaning weight has been found to influence both pup genders' survival rates during the first year (Hall *et al*., 2001)

It would seem that the female is responsible for placing a limit on expenditure during rearing, certainly with relation to nursing (Pomeroy *et al*., 1999), but possibly extending to other interactions. The limitation to nursing becomes more obvious in cases of allo-suckling, or in the rare occurrence of twins. Measurements made by Fogden (1971) show that mothers suckling two or more pups do not increase the total time spent suckling the pups compared to those with only one pup. Instead the same time is divided between the pups, reducing the nursing time for each individual pup but maintaining the total nursing time per cow. Maternal investment described in section 2.1.3.4 suggests that female grey seals have an inbuilt limit to the proportion of their stored resources they are willing to expend whilst ashore (usually around 46.5% of the maternal postpartum mass, Pomeroy *et al*., 1999). Past this limit, they may begin to risk their own reproductive success in the future by depleting their limited resources (Pomeroy *et al*., 1999).

A final explanation may be the restraint hypothesis described in section 2.1.3.4. This states that, in order to conserve reproductive energy for the future, a reduction in maternal effort is seen in younger individuals. The focal group at Donna Nook may encapsulate a wide range of age and experience. The variation in investment caused by age and experience may therefore mask any bias in maternal investment between male and female pups.

Unfortunately, no background data were available for the females at Donna Nook to provide either age or pupping experience, it is therefore impossible to identify any correlation between experience and maternal attendance behaviours. However, it is interesting at this point to note that female P20 from the public site is well known by the wardens at the reserve. She has a characteristic scar around her neck making her easily identifiable and has been returning to the same spot near the fence for a number of years to rear offspring. P20 has been listed as one of the four most affiliative mothers according to the MORQ index.

5.2.2 Regular Disturbance

It has been suggested that, for a number of pinniped species, the disturbance caused by human visitors at haul out sites can have significant impacts on behaviour (Fogden,

1971; Stevens & Boness, 2003; Engelhard *et al*., 2001). If this was indeed the case for grey seals at Donna Nook, behavioural time budgets should be different between the public site (open to visitors along a public footpath and experiencing several thousand visitors per week throughout the breeding season) and the RAF site (owned by the Ministry of Defence, cordoned off to the public).

On initial inspection of data provided by this study, the presence of humans on a daily basis throughout the breeding period appears to have minimal impact on maternal behaviours. During periods of directed mother-pup contact, the disturbance stimuli made no significant differences to any of the maternal behaviours analysed. During non-nursing periods, however, data suggested that mothers exposed to this regular disturbance on the public site spent a larger proportion of the observation time performing pup-check behaviours during late lactation. This significance did not extend to pup-check rates, however. Additionally, there was a trend towards more time spent playing during non-nursing periods in late lactation on the public site. Though this was not found to be significant, the p-value was close to the 5% confidence limit (Randomisation Statistic = 3.28, p = 0.06), thus the difference is worthy of further discussion. It was speculated in section 5.1.1 that the outcome of these play behaviours appear to be homologous to those brought about by licking and grooming in other mammals. Similarly, they may act to form and strengthen a bond between mother and pup during the breeding season. It would, therefore, appear that the presence of human disturbance is benefiting the pups by increasing interaction levels with their mothers. Again, though, we must consider whether increased maternal interaction is beneficial to pup development, or whether maternal attention will reduce exploration levels as described in guinea-pigs and primates. This is more difficult to apply to grey seals, during the early stages pups have little muscular control and so these mother-pup interactions may be slow to develop. Therefore we expect to see more of them during late lactation as the pup's co-ordination develops. However, this increase in play behaviour is not observed on the RAF site, suggesting there may indeed be a link between human disturbance and increased play behaviours.

The POF spent performing pup-check behaviours is significantly higher on the public site during late lactation than on the RAF site. Before discussing the implications of this finding, we must consider the data used to obtain it. Firstly, the data set is particularly small, 11 females on the RAF site had late random focal videos taken, but only 4 on the public site. The distribution of these videos with respect to the storm surge is given in **Table 5.2.1**, with half of late random focal videos on the public site being taken after the storm surge, but only 1 in 11

Table 5.2.1 Table showing the number of late lactation random focal videos taken during pre- and post-tidal periods

Tidal Period	Public	RAF
Pre-tidal	2	10
Post-tidal	2	1
Total	4	11

of those on the RAF site. The importance of this distribution is clear from looking at the results from the sudden disturbance analysis in which the POF spent performing pup-check behaviours significantly increases after the storm-surge. It is obvious from looking at **Table 5.2.2** that it is the results from the two females filmed after the storm surge that are providing the higher POF spent performing pup-checks on the public site. However, the two public site females with focal videos prior to the storm surge have higher POF spent performing pup-checks than the average value for the RAF site (2.86%). It may be possible, therefore, that there is an element of difference between the two sites. This would suggest that human disturbance is provoking an increase in pup-directed behaviours in grey seal mothers. Unlike the play behaviours, pup-checks do not, in themselves, increase contact between mother and pup and are therefore unlikely to cause any significant impact on the future behaviour of the offspring.

As mentioned previously, the presence of humans on the public site did not appear to make any significant differences to the time budgets of females during periods of directed mother-pup interaction. Donna Nook has a dedicated team of wardens patrolling the public walkway during the grey seal breeding season. In addition, two fences have been erected between the walkway and the colony (**Figure 5.2.1**). These fences are only around a meter high and do not provide the colony

Table 5.2.2 representing the percentage of focal spent on play and pup-check behaviours for mothers on the public site during late lactation; represents data collected after the storm surge (28/11/2011).

Seal ID	POF spent playing (%)	POF spent on pup-check (%)
P16	10.75	3
P21	1.18	4.34
P26	0	8.2
P36	5.4	7.57

Figure 5.2.1 Countryside type fence used at Donna Nook. Visitors were permitted on the walkway (left of the fence in above picture). Some seals pupped directly against the fence.

any protection from noise or visual disturbance from human visitors, it is also possible to cross them. However, in conjunction with the wardens, they act as a suggested barrier. A colony of South American fur seals (*Arctocephalus australis* Zimmermann, 1783) in Uruguay were separated from tourists using a similar "countryside type" fence. Cassini *et al*. (2004) compared behaviours on the colony the year before and the year after the fence was introduced. The presence of the fence reduced the intensity of response to disturbance, most noticeably a reduction in aggression and fleeing behaviour. There was also a reduction in behavioural responses to larger groups of visitors. However, over both years it was observed that the louder or more intrusive the human behaviour, the more intense the negative response it elicited from the colony (Cassini *et al*., 2004). It is possible that the presence of the fence is responsible, at least in part, for the behavioural similarities between females on the public site and those on the RAF site. Behaviour on colonies of harp seals (*Phoca goenlandica* Erxleben, 1777) in the Gulf of St Lawrence, Canada were compared between those exposed to tourism and those not (Kovacs & Innes, 1990). Non-exposed colony behaviours were used as a baseline for comparison of tourist colonies during and after bouts of visitors. During times of human disturbance, mothers spent a reduced amount of time with their pups. Those which remained with their pups showed a reduction in the time spent nursing, but an increase in the time spent alert. Pup behaviour changed, becoming more agonistic and less restful. However, these effects disappeared within one hour of the disturbance being removed, becoming comparable to the baseline behaviours (Kovacs & Innes, 1990). Some studies have been able to compare behaviour of the same individuals before, during and after the presence of humans. Engelhard *et al*. (2002) focussed on a colony of southern elephant seals on Macquarie Island. The colony was exposed to periods of human disturbance, both in the form of tourists and other researchers. Whilst humans were present, both the rate of maternal calls and alert behaviours amongst females increased significantly. However, these rates reduced

to normal quickly after the disturbance was removed (Engelhard *et al*., 2002). This produces interesting questions for the individuals on the public site at Donna Nook. Although visitor numbers fluctuated during the course of each day and peaked during the weekends, there was seldom, if ever, a prolonged period during daylight hours when the footpath was completely clear of humans. This means that, unlike the harp seals of St Lawrence or the elephant seals on Macquarie Island, the grey seals at Donna Nook do not experience a recovery period in which behavioural changes, triggered by disturbance, can be reversed. Consequently, we should expect to see disturbance behaviours (decreased maternal attendance, shorter and fewer suckling bouts, increased alertness) to be present consistently on the public site. This would likely be detrimental to survival of the pup, reducing protection available to the pup, and increasing the potential for starvation.

Clearly this is not the case at Donna Nook, the colony is thriving and increasing every year (Thompson & Duck, 2010). It is possible that the presence of disturbance at the public site, combined with the accessibility of the nearby, relatively undisturbed RAF site, places a selective pressure on individuals hauling out onto the colony with relation to pupping site. If an individual had a predisposed low tolerance to disturbance, pupping and breeding attempts on the public stretch of the colony may be relatively unsuccessful. Success may increase simply by selecting a pupping spot on the less disturbed RAF site. Those individuals with a higher tolerance towards disturbance may find adequate pupping and breeding success on the public site. This would explain the similarities in maternal behaviour between the two sites, as individuals on the RAF site would not experience the same levels of disturbance and those on the public site show higher tolerance and, as a result, minimal if any change in behaviour. Indeed, personal observations made during the field season suggest that this lack of response to human disturbance be an aspect of an overall lower response to disturbance. Individuals on the RAF site were exposed to a digger, driving through the colony every morning and evening. Responses to this were still extreme, with many individuals showing extended alert and pup-check behaviours, and in some cases approaching and aggressively displaying to the vehicle. On only a single occasion, a digger was driven directly through the colony on the public site. Despite having little if any prior experience of this disturbance, this section of the colony remained restful, with few if any alert behaviours (personal observation).

This observation lends weight toward human disturbance placing a pressure on pupping site selection. Another explanation for the similarity in behaviour between the disturbed and undisturbed stretches of the colony is

that individuals on the public site may have become habituated to the disturbance stimuli. Habituation has been defined as "response decrement as a result of repeated stimulation" (Harris, 1943; quoted from Thompson & Spencer, 1966). The diminution of the response can be anything from permanent to incredibly short-term, returning almost immediately after the stimulus stops (Thompson & Spencer, 1966). Depending on the permanency of the response decrement, habituation could take place either in the few days spent on the beach prior to pupping, or build up over a number of years due to site fidelity. The occurrence of habituation to human disturbance has been tested in Weddell seals (*Leptonychotes weddellii*) on breeding colonies in the Antarctic (Petel *et al*., 2008). Habituation was found to occur when human visits were made regularly within a short period of time. The total number of seals performing alert behaviours reduced from 67% to 18% by the 10th human visitation in the space of two hours. There was a reduction in the duration of these alert behaviours also. Irregular disturbance over several weeks did not lead to habituation (Petel *et al*., 2008). The habituation theory at Donna Nook is supported by Thompson and Duck (2010), who argue that the increase in colony size is evidence that human disturbance is not causing a negative impact on seal breeding.

5.2.3 Sudden Disturbance

The storm surge on November 28th 2011 caused a sudden and unexpected disturbance to the colony across both sites at Donna Nook. Many mother-pup pairs were separated, in some cases permanently, and a number of pups were seen without a mother in the weeks following. Time spent alert increased (though insignificantly) after the storm surge. Alert rate was not included in this section of the study. The high variability within females in duration for each occurrence of an alert behaviour suggests that alert duration is responsive to the environment. For this reason, it was felt that overall alert duration would provide a more adequate reflection of environmental disturbance than alert rate. A very clear increase was observed in time spent performing pup-check behaviours. Pup-check rates from focal videos were not included in this analysis as it was felt that in-field counts represented these more suitably. In field pup-check rates showed a significant increase in response to the sudden disturbance. The locomotive response of individuals remaining on the colony after the storm surge was away from the tide-line. This should have lead to an increase in colony density, in which case increased pup-checking rates could be attributed to overcrowding. However, data from proximity maps suggest that this was not the case. The median number of individuals within a 5bl radius of focal females

Figure 5.2.2 Top: RAF colony 3 days prior to the storm surge; Bottom: RAF colony 6 days after the storm surge.

decreased from 9 to 7, and overall decrease was found to be significant. The drop in the number of individuals within a 5bl radius is clear from both the graph in chapter 4 (**Figure 4.3.13**), and the photograph in **Figure 5.2.2**. Lower population density across the colony can be explained by the proximity to the end of the breeding season (a number of pups had been weaned and their mothers returned to the sea) as well as a mother's response to disturbance. Cows which are permanently separated from their pup, or whose pup has died will often leave the colony almost immediately. This does not appear to affect her chances of mating for that season, as she may remain in the vicinity of the colony until she comes into oestrus (Fogden, 1971). Another factor, other than increased density, must be responsible for the increased vigilance behaviour. Site fidelity (returning to the same pupping site) and natal philopatry (returning to the birthplace to pup) has been linked to the production of kin associations; seals are able to recognise their neighbours, and these neighbours are often the same from year to year due to site fidelity (Pomeroy *et al*., 2000*b*; Pomeroy *et al*., 2005). The need for vigilance behaviours and aggressive interactions would be minimised by the presence of known neighbours, likely reducing resource expenditure and increasing rearing success and future fitness as a result (Pomeroy *et al*., 2005). Indeed, on North Rona, females pupping in locations on the colony where the highest relatedness was recorded had the greatest success in terms of pup mass and growth rate. This was not linked to maternal mass or efficiency (Pomeroy *et al*., 2001). The disturbance and subsequent locomotion brought about by the storm surge at Donna Nook caused a change in position for many mother-pup pairs (personal observation). Thus, a sudden change in neighbours may

account for this increase in vigilance behaviours. Though no significant difference was observed in mean levels of PINT or play from pre-tidal to post-tidal periods, it is clear from the box-plot in **Figure 4.3.9** that the range of data for PINT was far larger pre-tidal, showing a sudden and obvious decrease after the storm surge. This is a very interesting response. Though it may simply be a reflection of the reduced sample size (see table 4.3.4), it could also suggest that between-individual variation in mother-pup interaction decreased suddenly after the storm surge. Again, this may be a result of new neighbours, causing a shift in the priority of behaviour from pup interaction to vigilance and aggression toward new adults. Certainly the former is supported by the current study.

When compared across the whole breeding season, no differences are found in alert duration between the public and RAF sites. However, when separated into pre-tidal and post-tidal periods we begin to see a pattern appearing. Prior to the storm surge, females on the RAF site spent a significantly greater duration of time alert than those on the public site. Again this may be attributed to the reasons given in section 5.2.2, either site selection or habituation. The former (that females pupping on the public site have a predisposed higher tolerance to disturbance) may, on initial inspection, appear to better account for this difference. However, alert duration on the public site more than doubles after the storm surge, leading to an equal alert duration between the public and RAF sites. This suggests that the tolerance to disturbance is reversible, and is therefore more likely due to habituation. If this is the case, it will likely be beneficial for individuals emigrating from nearby colonies, such as the Isle of May. This reversal of habituation suggests an element of flexibility in the tolerance to disturbance; individuals are not required to be excessively tolerant in order to pup successfully on the public site, but instead can habituate to the disturbance and respond to disturbance only when necessary. This may be the key to the success of the Donna Nook colony. An alternative explanation may be that females on the public site are capable of habituating to repeated disturbance but that this habituation is simply not transferrable to other disturbance situations.

5.3 LIMITATIONS OF AND POSSIBLE FUTURE EXTENSIONS TO THE CURRENT STUDY.

5.3.1 Limitations

5.3.1.1 Data collection

An obvious limitation comes from having access to only one breeding season at Donna Nook. This has prevented the use of the term 'mothering styles' in response to the appearance of CIDs in maternal behaviours. However, this study provides preliminary evidence of mothering styles in mother-pup interactions, leading a clear path for future research.

Data collection was limited to observations only. Weights were not collected, from either mother or pup. Previous research relies heavily on these physical measurements to determine maternal expenditure and the importance of this on the development of the pup. Similarly, age and experience data was not available for the females included in the study, nor post-weaning information on the pups of known mothers. For these reasons, links between maternal behaviour identified in this study to the prior experience of mothers and/or potential impacts on pup development can only be postulated at this stage; comparisons cannot be formed conclusively with previous literature. This observation method limited the pup gender record as gender identification was not always possible at a distance or from the angle.

The nature of the focal videos places limitations on the calculation of behaviour duration. During a 30 minute focal, an increased time spent on one behaviour decreases the time available for others. Certain behaviours, in particular play behaviours, occur infrequently throughout the day. A single 30 minute video focal is therefore unlikely to provide a true reflection of time spent on play. This method provided enough data for preliminary investigation into CIDs at Donna Nook, however any future attempts to further this research may benefit from using short-interval scan samples throughout the day, in place of the focal videos, in order to maximise behavioural data collected. The methods use required data to be collected from 3 to 5 individuals per day. For this reason, it would be possible to record behaviour from all individuals using 30 second scan samples. This would ensure a short enough interval that behaviours lasting only a few seconds (such as alert and pup-check) could still identified, but would provide an opportunity to spread data collection throughout the day, increasing the likelihood of capturing rare play behaviours. In addition to scan samples, in-field pup-check counts should be included in future research, as the data collected from these was invaluable to the results of the current study. Thus, time in the field could be spent alternating between one hour of 30-

second scan samples and one hour of pup-check counts throughout the day.

5.3.1.2 Study Site

Seals across the colony, both on the public and RAF sites and those in between, were exposed to military vehicle flyovers on numerous occasions. Because of the location of the study site, this was unavoidable but the impact on the study was minimised by the colony-wide spread of the disturbance. The colony on the RAF site was disturbed twice daily by the passage of a digger, providing transport between the military buildings to the left of the study area (shown as white block in satellite image in **Figure 3.1.1**) and the quad to the right of the study area. Visual effects of the disturbance on seal behaviour appeared to be limited to times when the disturbance was present. As such, impact on the study was minimised simply by not including focal videos interrupted by the digger.

Previous studies identifying the impacts of visitor disturbance on maternal behaviour and pup development have been able to compare the same individuals before, during and after the disturbance (Kovacs & Innes, 1990; Engelhard *et al.*, 2002). For this to be possible, an assured period of time without human disturbance is required, something which was never possible at Donna Nook. Being a public footpath and an area of high tourist volume, there is almost always human presence from dawn to dusk during the breeding season. Therefore, comparisons of an individual female's behavioural change due to disturbance could not be carried out. However, having access to the RAF stretch somewhat mitigates this limitation as, although it does not provide detail on the individuals' responses to disturbance, it does provide an excellent comparison with a relatively undisturbed stretch of the same colony, experiencing the same environmental conditions.

5.3.2 Future extensions to the current research

This study aimed to identify CIDs in maternal behaviours. This was designed to act as a springboard for future research, to enable conclusive identification of mothering styles and to monitor the impacts of these differing styles on pup development and future behaviour. A simple extension, and one which could assist in conclusively identifying mothering styles, would be to repeat the same study over a number of breeding seasons. If CIDs could be identified in mothering styles over a number of pupping periods, the possibility of pup-influenced consistent behaviour could be removed. This is one of the first studies carried out at Donna Nook in which females were individually identifiable by photo ID. The production of this photo-catalogue would allow long term records to be built over a number of years. This could begin to provide information on the prior experience of the females.

By combining data collection on females and their pups, it would be possible to determine what 'stimulus', if any, a pup presents to its mother and how the mother responds to this. CIDs could then be tested for in maternal response to pup stimulus to identify whether some mothers have a predisposition to greater pup-interaction than others.

The most exciting and interesting extensions come from identifying impacts of maternal behaviour on pup development. Behavioural observations used in this study could be combined with weight measurements from both mother and pup throughout rearing. This would provide an opportunity for comparison of maternal behaviour and pup weaning condition (thought to effect survival rate). More specific links could be drawn between maternal behaviour and pup development. During this discussion, play behaviours have been identified as potential homologues to the licking and grooming behaviours seen in other mammals. Licking and grooming has been linked to nursing bouts as well as increased offspring movement and body temperature (Champagne *et al*., 2007). Observations made during the current study suggest that play behaviours, too, appear to bring about nursing and offspring movement. An extension to this would be to produce behavioural data in a timeline. This could be used to assess the regularity with which play is associated with nursing bouts. Body temperature measurements, pre- and post-play, could be taken from pups to examine this impact. The use of satellite tagging, whilst costly and technically challenging, could prove to be enormously informative. Pups could be tagged, after their initial lanugo moult, to provide information on their survival and behaviour during the following year (until their next moult). Tagging can take two forms, satellite telemetry (giving detail of an individual's position using a GPS system) or sensor networks (providing the same information as well as contacts made and social networks formed between tagged individuals). The former would allow only known individuals to be tagged; satellite tags are able to remotely transmit their data back ashore (for example, using an Argos satellite modem; Vincent *et al*., 2002; Lindgren *et al*., 2008). The latter would require numerous pups to be tagged, not just those of known mothers. However, the majority of these tags could be inexpensive, local communication tags, exchanging information only with other tags in the nearby area. A small number of 'sink' tags could then be attached to fixed structures around the haul out area to obtain information from these tags to be collected for analysis. A protocol for using this within seal colonies has already been suggested - the Seal2Seal (S2S) protocol (Lindgren *et al*., 2008). Tagging could provide invaluable information about the exploratory tendencies of known pups, as well as the number of social contacts they make. This could then be linked to the mothering style experienced by that pup to determine whether, as noted in

Guinea pigs and primates, a relationship rich in maternal interactions has a negative effect on future pup exploration.

6. CONCLUSION

Around nursing bouts, grey seal mothers on the breeding colony at Donna Nook display consistent individual differences (CIDs) with regard to time spent interacting with their pup. Pup-check rates showed CIDs, in agreement with prior literature (Twiss *et al*., 2011; Twiss *et al*., 2012). In addition, CIDs were observed in mother-offspring relationship quality (MORQ) index values; affiliative mothers were consistently so from early to late lactation. There is no link, however, between an affiliative relationship and a high rate of pup-checking, suggesting that an affiliative relationship does not necessarily represent an overprotective mother. Mothers at Donna Nook spent a greater percentage of time interacting with their pup than those on the Isle of May (Kovacs, 1987; Twiss *et al*., 2000), suggesting that the costs of accessing the sea at Donna Nook are sufficient to prevent mothers from returning before their pup is weaned and that more time is therefore available for attendance to the pup.

Contrary to the suggestion by some authors that grey seal mothers of male pups should invest more into rearing than those of female pups, pup gender did not appear to impact upon maternal time budgets for the behaviours included in this study. Energetic investment was not considered here, however.

This study provides evidence to suggest that grey seal mothers are able to habituate to regular human disturbance. Despite visitor numbers in the tens of thousands, there was little evidence to suggest any difference in maternal time budgets between mothers on the public site and those on the RAF site (with the exception of alert duration prior to the storm surge). Expected behavioural changes in response to this disturbance (increased alert behaviours, decreased interaction with pup) were not observed amongst mothers at Donna Nook. Results suggested a higher duration of pup-checking during late lactation on the public site compared to the RAF site. This data must be interpreted with caution as 50% of late lactation data on the public site was collected after the storm surge, after which pup-check duration increased for the colony as a whole.

Once data was separated into pre- and post-tidal periods, it became clear that females on the public site spent significantly less time alert than those on the RAF site prior to the tidal storm surge. After the surge, however, average alert duration on the public site more than doubled and this difference was no longer apparent. This suggests that, if individuals on the public site have indeed habituated to public presence, this habituation is reversible on experiencing a novel, or at least irregular, disturbance. The duration of each individual alert behaviour

showed large intra-individual variation. Both duration and rate of alert behaviours also showed no CIDs. All of these factors combined may suggest that the performance of alert behaviours may be directly as a response to the environment, rather than an ingrained behaviour. This is contrary to findings based on male grey seals, for whom evidence of CIDs was found for alert behaviours (Twiss & Franklin, 2010). This difference may be due to the nature of the sampling used in the current study, recording only 30 minutes of a female's day.

Perhaps the most interesting finding of the current study was the increase in pup-check duration after the storm surge. This increase was apparent on both the public and RAF sites. This could not be attributed to a higher density of individuals, as the number of individuals within five body lengths of focal females significantly decreased after the storm surge. Instead it is possible that the rearrangement of positions among the colony brought about by the encroaching tide caused mothers to no longer be positioned near to known neighbours. This further supports the importance of kinship formations described by Pomeroy *et al*., (2000*b* and 2001).

A combination of public access restriction, enforced both by the fence and the dedicated efforts of the team of wardens, along with the species own ability to habituate to human disturbance appear to have prevented unnecessary maternal vigilance behaviours or decreased mother-pup interactions as a response to regular disturbance. The sudden increase in pup-check and alert duration in the weeks following the storm surge suggest that this habituation does not extend to unforeseen disturbance, and as a result should not impact upon the individuals' abilities to respond to threatening situations. This reversible habituation should allow individuals on the public stretch of the colony to pup on another colony in which disturbance posed a serious threat, and be capable of responding to this disturbance appropriately.

This study provides preliminary evidence for the existence of mothering styles in grey seals, adding to ever growing evidence for personalities in non-human animals. Investigation into the behaviours of the same females over subsequent lactation periods would be necessary to further support this. Human disturbance, with suitable management, does not appear to affect mother-pup interactions. Data on pup survival and behaviour into adulthood would be of use in addition to maternal data, to determine whether regular human disturbance at an early life stage has an effect on pup development. The results speak positively for the future of the Donna Nook breeding colony. Nevertheless, the continued success may well be dependent on continued restriction of the public, enforced by the warden team and fence. This will ensure that human disturbance remains as unobtrusive as possible.

REFERENCES

Abt, K. and Engler, J. (2009) Rapid increase of the grey seal (*Halichoerus grypus*) breeding stock at Helgoland. *Helgoland Marine Research*, **63**: 177-180.

Adriaenssens, B. and Johnsson, J.I. (2011) Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. *Behavioural Ecology*, **22**: 135-143.

Albers, P.C.H., Timmermans, P.J.A. and Vossen, J.M.H. (1999) Evidence for the existence of mothering styles in Guinea pigs (*Cavia aperea f. porcellus*). *Behaviour*, **136**: 469-479.

Albers, P.C.H., Timmermans, P.J.A. and Vossen, J.M.H. (2000*a*) Is maternal behaviour correlated with later explorative behaviour of young guinea pigs (*Cavia aperea f. porcellus*)? *Acta Ethology*, **2**: 91-96

Albers, P.C.H., Timmermans, P.J.A. and Vossen, J.M.H. (2000*b*) Effects of frequency and length of separation bouts between mother and offspring on later explorative behaviour of young Guinea-pigs (*Cavia aperea f. porcellus*). *Behaviour*, **137**: 1487-1502.

Anderson, S.S. and Fedak, M.A. (1985) Grey seal males: energetic and behavioural links between size and sexual success. *Animal Behaviour*, **33**: 829-838.

Anderson, S.S. and Fedak, M.A. (1987) Grey seal, *Halichoerus grypus*, energetics: females invest more in male offspring. *Journal of Zoology*, **211**: 667-679.

Arnbom, T., Fedak, M.A. and Boyd, I.L. (1997) Factors affecting maternal expenditure in southern elephant seals during lactation. *Ecology*, **78**: 471-483.

Bardi, M. and Huffman, M.A. (2002) Effects of maternal style on infant behaviour in Japanese macaques (*Macaca fuscata*). *Developmental Psychobiology*, **41**: 364-372.

Bardi, M. and Huffman, M.A. (2006) Maternal behaviour and maternal stress are associated with infant behavioural development in macaques. *Developmental Psychobiology*, **48**: 1-9.

Barnard, C.J. and Sibly, R.M. (1981) Producers and scroungers: A general model and its application to captive flocks of house sparrows. *Animal Behaviour*, **29**: 543-550.

Bekoff, M. (1972) The development of social interaction, play and metacommunication in mammals: an ethological perspective. *The Quarterly Review of Biology*, **47**: 412-434.

Bell, A.M., Hankison, S.J. and Laskowski, K.L. (2009) The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, **77**: 771-783

Bergmüller, R. and Taborsky, M. (2010) Animal personality due to social niche specialisation. *TRENDS in Ecology and Evolution*, **25**: 504-511.

Boness, D.J. (1984) Activity budget of male gray seals, *Halichoerus grypus*. *Journal of Mammalogy*, **65**: 291-297.

Bowen, W.D., McMillan, J. and Mohn, R. (2003) Sustained exponential population growth of grey seals at Sable Island, Nova Scotia. *ICES Journal of Marine Science*, **60**: 1265-1274.

Bowen, W.D., Oftedal, O.T., Boness, D.J. and Iverson, S.J. (1993) The effect of maternal age and other factors on birth mass in the harbour seal. *Canadian Journal of Zoology*, **72**: 8-14.

Bowen, W.D., Ellis, S.L., Iverson, S.J. and Boness, D.J. (2001) Maternal effects on offspring growth rate and weaning mass in harbour seals. *Canadian Journal of Zoology*, **79**: 1088-1101.

Bowen, W.D., Iverson, S.J., McMillan, J.I. and Boness, D.J. (2006) Reproductive performance in grey seals: age-related improvement and senescence in a capital breeder. *Journal of Animal Ecology*, **75**: 1340-1351.

Brace, N., Kemp, R. and Snelgar, R. (2002) Chapter 7: Multiple Regression. In: *SPSS for Psychologists*. London: Palgrave MacMillan. 206-220.

Caldji, C., Diorio, J. and Meaney, M.J. (2000) Variations in maternal care in infancy regulate the development of stress reactivity. *Biological Psychiatry*, **48**: 1164-1174.

Cassini, M.H., Szteren, D. and Fernández-Juricic, E. (2004) Fence effects on the behavioural responses of South American fur seals to tourist approaches. *Journal of Ethology*, **22**: 127-133.

Caudron, A.K., Kondakov, A.A. and Siryanov, S.V. (1998) Acoustic structure and individual variation of grey seal (*Halichoerus grypus*) pup calls. *Journal of the Marine Biological Association UK*, **78**: 651-658.

Champagne, F.A. and Meaney, M.J. (2007) Transgenerational effects of social environment on variations in maternal care and behavioural response to novelty. *Behavioural Neuroscience*, **121**: 1353-1363.

Champagne, F.A., Curley, J.P., Keverne, E.B. and Bateson, P.P.G. (2007) Natural variations in postpartum maternal care in inbred and outbred mice. *Physiology & Behaviour*, **91**: 325-334.

Coulson, J.C. and Hickling, G. (1964) The breeding biology of the grey seal, *Halichoerus grypus* (Fab.), on the Farne Islands, Northumberland. *Journal of Animal Ecology*, **33**: 485-512.

Dall, S.R.X., Houston, A.I. and McNamara, J.M. (2004) The behavioural ecology of personality: consistent individual

differences from an adaptive perspective. *Ecology Letters*, **7**: 734-739.

DeWitt, T.J., Sih, A. and Wilson, D.S. (1998) Costs and limits of phenotypic plasticity. *TRENDS in Ecology and Evolution*, **13**: 77-81

Dingemanse, N.J. and Réale, D. (2005) Natural selection and animal personality. *Behaviour*, **142**: 1159-1184.

Dingemanse, N.J. and Wolf, M. (2010) Recent models for adaptive personality differences: a review. *Phil. Trans. R. Soc. B*, **365**: 3947-3958.

Dingemanse, N.J., Both, C., Drent, P.J. and Tinbergen, J.M. (2004) Fitness consequences of avian personalities in a fluctuating environment. *Proc. R. Soc. Lond.* B, **271**: 847-852.

Dingemanse, N.J., Kazem, A.J.N., Réale, D. and Wright, J. (2010) Behavioural reaction norms: animal personality meets individual plasticity. *TRENDS in Ecology and Evolution*, **25**: 81-89.

Dunn, J.F. (1975) Consistency and change in styles of mothering. *Parent-infant Interactions*, CIBA Foundation Symposium 33, Elsevier, Amsterdam; 155-176.

Engelhard, G.H., van den Hoff, J., Broekman, M., Baarspul, A.N.J., Field, I., Burton, H.R. and Reijnders, P.J.H. (2001) Mass of weaned elephant seal pups in areas of low and high human presence. *Polar Biology*, **24**: 244-251.

Engelhard, G.H., Baarspul, A.N.J., Broekman, M., Creuwels, J.C.S. and Reijnders, P.J.H. (2002) Human disturbance, nursing behaviour and lactational pup growth in a declining southern elephant seal (*Mirounga leonina*) population. *Canadian Journal of Zoology*, **80**: 1876-1886.

Fogden, S.C.L. (1971) Mother-young behaviour at grey seal breeding beaches. *Journal of Zoology*, **164**: 61-92.

Francis, D., Diorio, J., Liu, D. and Meaney, M.J. (1999) Nongenomic transmission across generations of maternal behaviour and stress responses in the rat. *Science, New Series*, **286**: 1155-1158.

Gosling, S.D. (2001) From Mice to Men: What can we learn about personality from animal research? *Psychological Bulletin,* **127**: 45-86.

Gosling, S.D. (2008) Personality in non-human animals. *Social and Personailty Psychology Compass*, **2**: 985-1001.

Gray, C.M., Brooke, M.L. and Hamer, K.C. (2005) Repeatability of chick growth and food provisioning in Manx shearwaters *Puffinus puffinus*. *Journal of Avian Biology*, **36**: 374-379.

Hall, A.J., McConnell, B.J. and Barker, R.J. (2001) Factors affecting first-year survival in grey seals and their implications for life history strategy. *Journal of Animal Ecology*, **70**: 138-149.

Hill, H.M., Greer, T., Solangi, M. and Kuczaj II, S.A. (2007) All mothers are not the same: maternal styles in bottlenose dolphins (*Tursiops truncatus*). *International Journal of Comparative Psychology*, **20**: 35-54.

Hopper, K.R., Rosenheim, J.A., Prout, T. and Oppenheim, S.J. (2003) Within-generation bet hedging: a seductive explanation? *Oikos*, **101**: 219-222.

Insley, S.J., Phillips, A.V. and Charrier, I. (2003) A review of social recognition in pinnipeds. *Aquatic Mammals*, **29**: 181-201.

Iverson, S.J., Bowen, W.D., Boness, D.J. and Oftedal, O.T. (1993) The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). *Physiological Zoology*, **66**: 61-88.

Kastelein, R.A., Wiepkema, P.R. and Vaughan, N. (1991) The suckling period of a Grey seal (*Halichoerus grypus*) while the mother had access to a pool. *Aquatic Mammals*, **17**: 42-51.

King, O.D. and Masel, J. (2007) The evolution of bet-hedging adaptations to rare scenarios. *Theoretical Population Biology*, **72**: 560-575.

Kovacs, K.M. and Innes, S. (1990) The impact of tourism on harp seals (*Phoca groenlandica)* in the Gulf of St. Lawrence, Canada. *Applied Animal Behaviour Science*, **26**: 15-26.

Kovacs, K.M. (1987) Maternal behaviour and early behavioural ontogeny of grey seals (*Halichoerus grypus*) on the Isle of May, UK. *Journal of Zoology*, **213**: 697-715.

Kurvers, R.H.J.M., van Santen de Hoog, S.I., van Wieren, S.E., Ydenberg, R.C. and Prins, H.H.T. (2012) No evidence for negative frequency-dependent feeding performance in relation to personality. *Behavioural Ecology*, **23**: 51-57.

Lee, P.C. (1984) Early infant development and maternal care in free-ranging Vervet monkeys. *Primates*, **25**: 36-47.

Lincolnshire Wildlife Trust. (2012). *Nature Reserve Maps: Donna Nook*. Available: http://lincstrust.org.uk/reserves/nr/reservemaps.php?mapref=15. Last accessed 11th May 2012.

Lindgren, A., Mascolo, C., Lonergan, M. and McConnell, B. (2008) Seal-2-Seal: A delay-tolerant protocol for contact logging in wildlife monitoring sensor networks. In *Proceedings of IEEE International Conference on Mobile Ad-hoc and Sensor Systems (MASS '08)*: 321-327.

Luttbeg, B. and Sih, A. (2010) Risk, resources and state-dependent adaptive behavioural syndromes. *Phil. Trans. R. Soc.* B, **365**: 3977-3990.

Maestripieri, D., Hoffman, C.L., Anderson, G.M., Carter, C.S. and Higley, J.D. (2009) Mother-infant interactions in free-ranging rhesus macaques: relationships between physiological and behavioural variables. *Physiology & Behaviour*, **96**: 613-619.

McElreath, R. and Strimling, P. (2006) How noisy information and individual asymmetries can make 'personality' an adaptation: a simple model. *Animal Behaviour*, **72**: 1135-1139.

Meaney, M.J. (2001) Maternal care, gene expression and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience*, **24**: 1161-1192.

Mellish, J.E., Iverson, S.J. and Bowen, W.D. (1999) Variation in milk production and lactation performance in grey seals and consequences for pup growth and weaning characteristics. *Physiological and Biochemical Zoology*, **72**: 677-690.

Nakagawa, S., Gillespie, D.O.S., Hatchwell, B.J. and Burke, T. (2007) Predictable males and unpredictable females: sex difference in repeatability of parental care in a wild bird population. *Journal of Evolutionary Biology*, **20**: 1674-1681.

Nesse, R.M. (2001) The smoke detector principle. Natural selection and the regulation of defensive responses. *Annals of the New York Academy of Sciences*, **935**: 75-85.

Nesse, R.M. (2005) Natural selection and the regulation of defenses. A signal detection analysis of the smoke detector principle. *Evolution and Human Behaviour*, **26**: 88-105.

Pavlov, I.P. (1960) *Conditioned Reflexes*. London: Oxford University Press.

Petel, T.P., Giese, M. and Hindell, M. (2008) A preliminary investigation of the effect of repeated pedestrian approaches to Weddell seals (*Leptonychotes weddellii*). *Applied Animal Behaviour Science*, **112**: 205-211.

Pomeroy, P.P., Anderson, S.S., Twiss, S.D. and McConnell, B.J. (1994) Dispersion and site fidelity of breeding female grey seals (*Halichoerus grypus*) on North Rona, Scotland. *Journal of Zoology*, **233**: 429-447.

Pomeroy, P.P., Fedak, M.A., Rothery, P. and Anderson, S. (1999) Consequences of maternal size for reproductive expenditure and pupping success of grey seals at North Rona, Scotland. *Journal of Animal Ecology*, **68**: 235-253.

Pomeroy, P.P., Twiss, S.D. and Duck, C.D. (2000*a*) Expansion of a grey seal (*Halichoerus grypus*) breeding colony: changes in pupping site use at the Isle of May, Scotland. *Journal of Zoology,* **250**: 1-12.

Pomeroy, P.P., Twiss, S.D. and Redman, P. (2000*b*) Philopatry, site fidelity and local kin associations within grey seal breeding colonies. *Ethology*, **106**: 899-919.

Pomeroy. P.P., Wilmer, J.W., Amos, W. and Twiss, S.D. (2001) Reproductive performance links to fine-scale spatial patterns of female grey seal relatedness. *Proc. R. Soc. Lond.* B, **268**: 711-717.

Pomeroy, P.P., Redman, P.R., Ruddell, S.J.S., Duck, C.D. and Twiss, S.D. (2005) Breeding site choice fails to explain interannual associations of female grey seals. *Behavioural Ecology and Sociobiology*, **57**: 546-556.

Pomeroy, P.P., Smout, S., Moss, S., Twiss, S.D. and King, R. (2010) Low and delayed recruitment at two grey seal breeding colonies in the UK. *Journal of Northwest Atlantic Fishery Science*, **42**: 125-133.

Potti, J., Moreno, J. and Merino, S. (1999) Repeatability of parental effort in male and female Pied Flycatchers as measured with doubly labeled water. *Canadian Journal of Zoology*, **77**: 174-179.

Rands, S.A., Cowllshaw, G., Pettifor, R.A., Rowcliffe, J.M. and Johnstone, R.A. (2003) Spontaneous emergence of leaders and followers in foraging pairs. *Nature*, **423**: 432-434.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. and Dingemanse, N.J. (2007) Integrating animal temperament within ecology and evolution. *Biological Review*, **82**: 291-318.

Reddon, A.R. (2012) Parental effects on animal personality. *Behavioural Ecology,* **23**: 242-245.

Redman, P., Pomeroy, P.P. and Twiss, S.D. (2001) Grey seal maternal attendance patterns are affected by water availability on North Rona, Scotland. *Canadian Journal of Zoology*, **79**: 1073-1079.

Schwagmeyer, P.L. and Mock, D.W. (2003) How consistently are good parents good parents? Repeatability of parental care in the house sparrow, *Passer domesticus*. *Ethology*, **109**: 303-313.

Sih, A., Bell, A. and Johnson, J.C. (2004) Behavioural syndromes: an ecological and evolutionary overview. *TRENDS in Ecology and Evolution*, **19**: 372-378.

Sih, A. (2011). Behavioural Syndromes: A behavioural ecologist's view on the evolutionary and ecological implications of animal personalities. In: Weiss, A., King, J.E. and Murray, L. *Personality and temperament in nonhuman primates*. New York: Springer, New York. 313-336.

Smiseth, P.T. and Lorentsen, S. (1995*a*) Evidence of equal maternal investment in the sexes in the polygynous and sexually dimorphic grey seal (*Halichoerus grypus*). *Behavioural Ecology and Sociobiology*, **36**: 145-150.

Smiseth, P.T. and Lorentsen, S. (1995*b*) Behaviour of female and pup grey seals *Halichoerus gypus* during the breeding period at Froan, Norway. *Journal of Zoology*, **236**: 11-16.

Smiseth, P.T. and Lorentsen, S. (2001) Begging and parent-offspring conflict in grey seals. *Animal Behaviour*, **62**: 273-279.

Smith, J.M. (1982) *Evolution and the Theory of Games*. Cambridge: Cambridge University Press.

Stamps, J.A. and Groothuis, T.G.G. (2010) The development of animal personality: relevance, concepts and perspectives. *Biological Reviews*, **85**: 301-325.

Stamps, J.A. (2007) Growth-mortality tradeoffs and 'personality traits' in animals. *Ecology Letters*, **10**: 355-363.

Stevens, M.A. and Boness, D.J. (2003) Influences of habitat features and human disturbance on use of breeding sites by a declining population of southern fur seals (*Arctocephalus australis*). *Journal of Zoology (London)*, **260**: 145-152.

Thompson, D., & Duck, C. (2010). Berwickshire and North Northumberland Coast European Marine Site: grey seal population status. *Report to Natural England*.

Thompson, R.F. and Spencer, W.A. (1966) Habituation: a model phenomenon for the study of neuronal substrates of behaviour. *Psychological Review*, **73**: 16-43.

Tinker, M.T., Kovacs, K.M. and Hammill, M.O. (1995) The reproductive behaviour and energetics of male gray seals (*Halichoerus grypus*) breeding on a land-fast ice substrate. *Behavioural Ecology and Sociobiology*, **36**: 159-170.

Twiss, S.D. and Franklin, J. (2010) Individually consistent behavioural patterns in wild, breeding male grey seals (*Halichoerus grypus*). *Aquatic Mammals,* **36**: 234-238.

Twiss, S.D., Caudron, A., Pomeroy, P.P., Thomas, C.J. and Mills, J.P. (2000) Finescale topographical correlates of behavioural investment in offspring by female grey seals, *Halichoerus grypus*. *Animal Behaviour*, **59**: 327-338.

Twiss, S.D., Wright, N.C., Dunstone, N., Redman, P., Moss, S. and Pomeroy, P.P. Behavioural evidence of thermal stress from overheating in UK breeding gray seals. *Marine Mammal Science*, **18**: 455-468.

Twiss, S.D., Poland, V.F., Graves, J.A. and Pomeroy, P.P. (2006) Finding fathers: spatio-temporal analysis of paternity assignment in grey seals (*Halichoerus grypus*). *Molecular Ecology*, **15**: 1939-1953.

Twiss, S.D., Culloch, R. and Pomeroy, P.P. (2011) An in-field experimental test of pinniped behavioural types. *Marine Mammal Science*, **28**: 280-294.

Twiss, S.D., Cairns, C., Culloch, R.M., Richards, S.A. and Pomeroy, P.P. (2012) Variation in female grey seal (*Halichoerus grypus*) reproductive performance correlates to proactive-reactive behavioural types. *PLoS ONE*, **7**: 1-11

Vincent, C., McConnell, B.J., Ridoux, V. and Fedak, M.A. (2002) Assessment of ARGOS location accuracy from satellite tags deployed on captive grey seals. *Marine Mammal Science*, **18**: 156-166.

Watters, J.V. and Powell, D.M. (2012) Measuring animal personality for use in population management in zoos: suggested methods and rationale. *Zoo Biology*, **31**: 1-12.

Weaver, A. and de Waal, F.B.M. (2002) An index of relationship quality based on attachment theory. *Journal of Comparative Psychology*, **116**: 93-106.

Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M. and Meaney, M.J. (2004) Epigenetic programming by maternal behaviour. *Nature Neuroscience*, **7**: 847-854.

Wilson, D.S., Clark, A.B., Coleman, K. and Dearstyne, T. (1994) Shyness and boldness in humans and other animals. *TRENDS in Ecology and Evolution*, **9**: 442 - 446.

Wilson, S. (1974) Juvenile play of the common seal *Phoca vitulina vitulina* with comparative notes on the grey seal *Halichoerus grypus*. *Behaviour*, **48**: 37-60.

Wolf, M. and Weissing, F.J. (2010) An explanatory framework for adaptive personality differences. *Phil. Trans. R. Soc.* B, **365**: 3959-3968.

Statistical Software

Microsoft (2010). Microsoft Excel [Computer software]. Redmond, Washington: Microsoft.

Minitab 16 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com)

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Todman, J.B. and Dugard, P. (2001). *Single case and small-n experimental designs*. Philadelphia: Taylor & Francis inc. Psychology press.